Agronomy Research

Established in 2003 by the Faculty of Agronomy, Estonian Agricultural University

Aims and Scope:
Agronomy Research is a peer-reviewed international Journal intended for publication of broad-spectrum original articles, reviews and short communications on actual problems of modern biosystems engineering incl. crop and animal science, genetics, economics, farm- and production engineering, environmental aspects, agro-ecology, renewable energy and bioenergy etc. in the temperate regions of the world.

***Agronomy Research* online:**
Agronomy Research is available online at: http://agronomy.emu.ee/

Acknowledgement to Referees:
The Editors of *Agronomy Research* would like to thank the many scientists who gave so generously of their time and expertise to referee papers submitted to the Journal.

Abstracted and indexed:
SCOPUS, EBSCO, CABI Full Paper and Thompson Scientific database: (Zoological Records, Biological Abstracts and Biosis Previews, AGRIS, ISPI, CAB Abstracts, AGRICOLA (NAL; USA), VINITI, INIST-PASCAL).

Subscription information:
Institute of Technology, EULS
St. Kreutzwaldi 56, 51014 Tartu, ESTONIA
E-mail: timo.kikas@emu.ee

Journal Policies:
Estonian University of Life Sciences, Latvia University of Life Sciences and Technologies, Vytautas Magnus University Agriculture Academy, Lithuanian Research Centre for Agriculture and Forestry, and Editors of *Agronomy Research* assume no responsibility for views, statements and opinions expressed by contributors. Any reference to a pesticide, fertiliser, cultivar or other commercial or proprietary product does not constitute a recommendation or an endorsement of its use by the author(s), their institution or any person connected with preparation, publication or distribution of this Journal.

ISSN 1406-894X

CONTENTS

Agronomy Research **17**(6), 2146–2157, 2019
https://doi.org/10.15159/AR.19.204

The efficiency of nitrogen fertilizer on the dry matter yield of tall fescue and festulolium grown as feedstock for combustion

A. Adamovics[1], R. Platace[1], S. Ivanovs[1,*] and I. Gulbe[2]

[1]Latvia University of Life Sciences and Technologies, Liela iela 2, LV-3001 Jelgava, Latvia
[2]Institute of Agricultural Resources and Economics, Struktoru iela 15, LV-2006 Riga, Latvia
[*]Correspondence: semjons@apollo.lv

Abstract. Grass biomass grows during one vegetation season and can be cultivated and consumed at the place of breeding. Grass biomass can be used not only in traditional feed, but, recently, also for energy production (biogas, solid fuels). The most important economic indicator for any crop is its productivity. The study found that it is important to use nitrogen fertilizer to increase the productivity of tall fescue and festulolium. A significant increase ($p < 0.05$) in the yield of tall fescue was observed starting from the nitrogen norm of 60 kg ha^{-1} N. Further increase in nitrogen fertilizer norm provides a significant increase in dry mater yield of tall fescue (reaching 8.64 t ha^{-1}) and festulolium (reaching 8.11 t ha^{-1}) at 180 kg ha^{-1} N. The analysis of linear regression coefficients of polynomials showed that the highest nitrogen efficiency in the first year of the use of tall fescue was achieved at the norm of 180 kg ha^{-1} N, but for festulolium – at the norm of 120 kg ha^{-1} N. In the following years of tall fescue use, the highest efficiency of nitrogen norms differed: in the 2nd and 4th year of use – at 60 kg ha^{-1} N, in the 3rd year of use – at 30 kg ha^{-1} N, and in the 5th year of use – at 120 kg ha^{-1} N. In contrast, for festulolium, in the 2nd year of use, the highest nitrogen efficiency was reached at the norm of 30 kg ha^{-1} N, and in the 3rd–5th year of use – at the norm of 60 kg ha^{-1} N.

Key words: tall fescue, festulolium, nitrogen fertilizer, dry matter yield, solid fuels.

INTRODUCTION

Grass biomass grows during one vegetation season and can be cultivated and consumed at the place of breeding. Grass biomass can be used not only in traditional feed, but, recently, also for energy production (biogas, solid fuels). The most important economic indicator for any crop is its productivity.

Many countries in Europe and other regions are dependent on fossil energy sources. In order to maintain a sufficient amount of energy supplies for future generations and less reliance on imports, there is a necessity to find a proper alternative to fossil fuels (Scholz & Ellerbrock, 2002; Lewandowski et al., 2003; Tonn et al., 2010; Heinsoo et al., 2011; Nilsson et al., 2015).

The growing of grass for the production of fuel has been a well-accepted technology in Europe but not in the Baltic States. Energy crops have been reported to be potential solid biofuels in Northern Europe (Kaķītis et al., 2009; Tilvikiene et al., 2016; Adamovics et al., 2017a).

The burning of grass pellets as a biofuel is economical, energy-efficient, environmentally friendly and sustainable (Tara et al., 2016).

The biofuels made from perennial grasses and other cellulosic biomasses could meet renewable fuel goals in the Baltic States, with little impact on food production and reducing the fossil fuel use and greenhouse gas emissions.

Nitrogen fertilizers play an important role in optimizing the grasses life processes, increasing the productivity, and improving the harvest quality. Nitrogen fertilizer significantly increases the yield and quality of grasses if the soil contains low levels of organic matter and nitrogen supply (Prochnow et al., 2009; Adamovics et al., 2017a). The dependence of terrestrial biomass yield on fertilizers is not unequivocal as several studies yield different results. Some studies have shown a linear dependence between nitrogen fertilizer rates and biomass increases (Landström et al., 1996; Scholz & Ellerbrock, 2002; Lemus et al., 2008; Mulkey et al., 2008; Adamovics et al., 2017b) while other nitrogen fertilizer norms do not affect biomass productivity and nitrogen content (Lewandowski & Kicherer, 1997).

The objective of the current study was to assess the influence of nitrogen fertilizer on the dry matter yield of grasses grown in five harvesting years as feedstock for the combustion and energy yield of perennial energy crops - festulolium and tall fescue.

MATERIALS AND METHODS

Field trials were conducted at the 'Peterlauki' Study and Research Farm (56°53' N, 23°71' E) of the Latvia University of Life Sciences and Technologies in 2011. The study was conducted from 2012 to 2016.

Soil characteristics: sod calcareous soil LUVISOLS (according to the FAO classification); granulometric composition: heavy dusty sand clay. Soil agrochemical parameters: pH_{KCL} 6.7 (LVS ISO 10390: 2006), organic matter content 21 g kg^{-1} (by Tyurin method; LV ST ZM 80-91), phosphorus content 52 mg kg^{-1} P_2O_5, and potassium content 128 mg kg^{-1} K_2O (according to the Egner-Rhym method; LV ST ZM 82-97).

Before grasses, summer barley was grown for the last two years. The study was carried out on festulolium (× *Festulolium* Asch. & Graebn.) cultivar 'Vetra' and tall fescue (*Festuca arundinaceae* Schreb.) cultivar 'Fawn'. After harvesting barley, the soil was ploughed 22 cm deep in October. In the spring, when the soil reached physical-mechanical readiness, it was first broken off (27.04.2011) and then cultivated (9.05.2011). The experimental plot was sown using the seed-drill 'Hēge-80' on 10 May 2011. Seed rate of 1000 germinating seeds per 1 m^2 was: for festulolium – 9.5 kg ha^{-1}, and for tall fescue – 17.0 kg ha^{-1}.

The fertilizers applied included: P80K120 kg ha^{-1} – main fertilizer (F), and six levels of additional nitrogen fertilizers: F + 30, F + 60, F + 90, F + 120, F + 150, and F + 180 kg ha^{-1} N. In the year of grass sowing, mineral fertilizers were not used but, instead, were applied in the spring of the years of grass use. On the field, fertilizers were applied by hand.

The harvested plot size was 10 m^2, with a 2-m wide column between grass species. The experiment was laid out in a randomized complete block design with three replicates. During the experiment, once in summer, weeding was carried out during the wake of grasses. Herbicides were not used in planting. The grasses harvesting time was the first cut at the end of July (the biomass after the first cut in July was harvested for the second time in mid-September, but the biomass yield of the second cut was not included in this assessment).

The total biomass yield was calculated using fresh weight and moisture content. The sample moisture level was measured after oven drying at 105 °C for 12 h.

Ash melting temperature was identified in the Waste and Fuel Research and Testing Laboratory 'Virsma' Ltd. using the methods of the standard ISO 540 LVS NE 15370-1. During the pellet manufacturing process, grass biomass was ground to fine powder with the electric mill 'ЭМ-3А УХЛ 4.2' in a laboratory of LLU. The obtained grass powder was formed into pellets using the hand press 'IKA WERKE'. The highest calorific value (Qa) was determined according to the standard ISO 1928 LVS EN 14918. The highest calorific value of germ and wood biomass was used to calculate the amount of energy produced. The energy value of pellets was calculated using the following formula (Kaķītis, Smits, Belicka, 2009):

$$Q_{kop} = Q_a \cdot M_s \tag{1}$$

where Q_{kop} – total amount of energy obtained from 1 ha, MJ ha^{-1}; Q_a – highest calorific value of biomass dry matter, MJ kg^{-1}; M_s – dry mass of the biomass of 1 hectare, kg ha^{-1}.

The average data of the experimental sites were statistically analysed using the three-way analysis of variance with 'grass species', 'fertiliser', and 'year of sward use' as factors, and the difference among means was detected by LSD at the $p < 0.05$ probability level (Excel for Windows, 2003).

Characteristics of meteorological conditions. For the period from 01/01/2011 to 31/12/2016, the data about the air temperature and rainfall were obtained from the automatic Meteorological station at the 'Peterlauki' Study and Research Farm of the Latvia University of Life Sciences and Technologies. For the comparison of annual meteorological data, the long-term average temperature and precipitation data (norm) were used from the Jelgava Meteorological station (Figs 1–2).

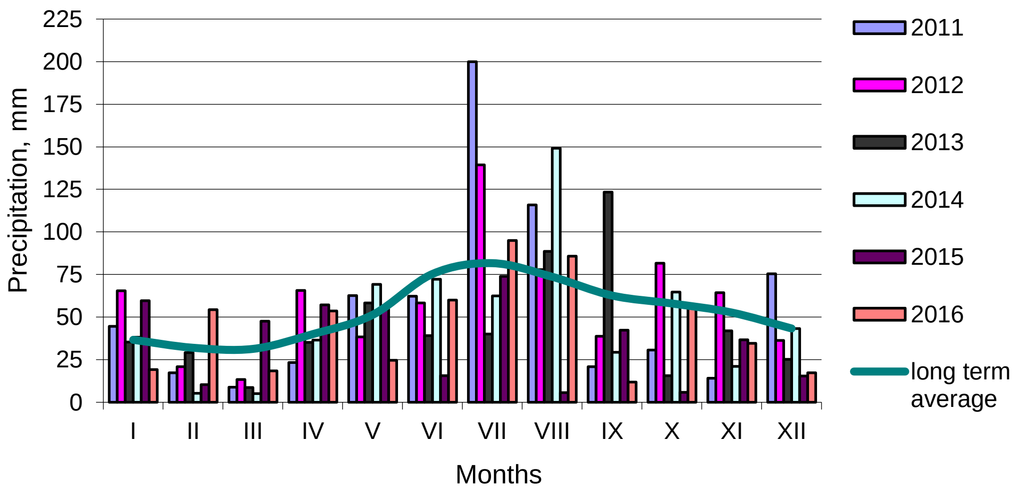


Figure 1. The average accumulated precipitation in 2011–2016.

Climatic conditions affect grass hibernation and growing of grassland and determine the size of the crop. The length of the wintering period varied from 128 days in 2013/2014 to 173 days in 2012/2013. During the five-year period, only two winters had a negative average air temperature. The coldest winter was in 2012/2013, when the average temperature dropped to -2.5 °C, whereas the warmest winters were in 2014/2015 and 2015/2016, when the average temperature in wintertime reached or exceeded +1.0 °C.

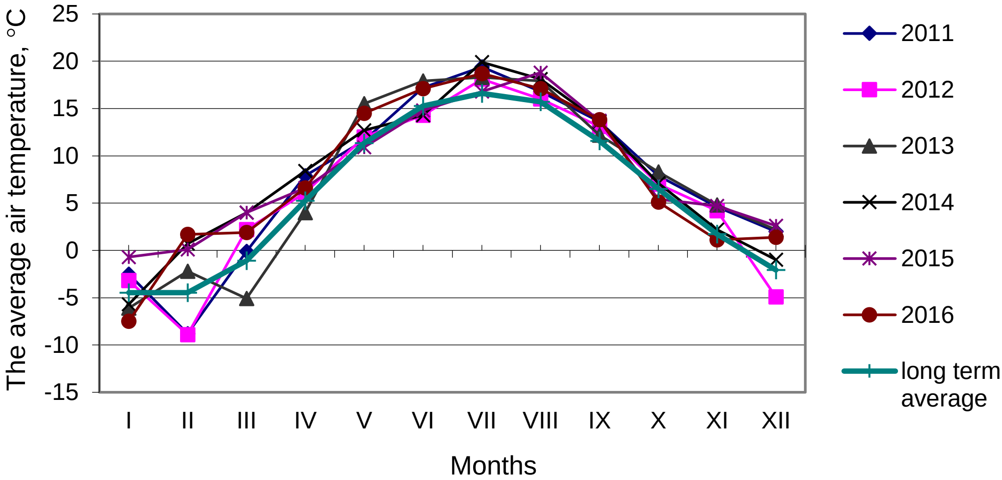


Figure 2. The average air temperature in 2011–2016.

In the vegetation period, the amount of precipitation corresponded to the norm in 2011, 2012 and 2014, when it was consistent with the average amount observed on a long-term basis; however, in 2013, 2015 and 2016, it was significantly lower than the norm.

Climatic conditions affect grass hibernation and growing of grassland and determine the size of the crop. The length of the wintering period varied from 128 days in 2013/2014 to 173 days in 2012/2013. During the five-year period, only two winters had a negative average air temperature. The coldest winter was in 2012/2013, when the average temperature dropped to -2.5 °C, whereas the warmest winters were in 2014/2015 and 2015/2016, when the average temperature in wintertime reached or exceeded +1.0 °C.

In the vegetation period, the amount of precipitation corresponded to the norm in 2011, 2012 and 2014, when it was consistent with the average amount observed on a long-term basis; however, in 2013, 2015 and 2016, it was significantly lower than the norm.

RESULTS AND DISCUSSION

The dry matter yield in the first year of festulolium use varied from 4.33 t ha^{-1} (N0P80K120) to 8.96 t ha^{-1} (N180P80K120), but for tall fescue – from 5.01 t ha^{-1} (N0P80K120) to 9.38 t ha^{-1} (N180P80K120) (Fig. 3). Compared to festulolium, tall fescue was more productive from the first to fourth year of use, as well as in variants where no nitrogen fertilizers were used; in other years of grass use, festulolium was more productive.

The results showed that tall fescue and festulolium were responsive to nitrogen fertilizers and the increase in nitrogen content also significantly increased the yield of dry matter.

The dry matter yield of tall fescue increased with the increase in nitrogen fertilizer norm: from 5.48 t ha^{-1} at 30 kg ha^{-1} N to 8.32 t ha^{-1} at 180 kg ha^{-1} N. On average, over the five years of the experiment, as a result of nitrogen fertilizer use, the dry matter yield increased from 22% (nitrogen norm 30 kg ha^{-1} N) to 81% (nitrogen norm 180 kg ha^{-1} N). In the variant with the lowest nitrogen norm (30 kg ha^{-1} N), the dry matter yield of festulolium increased significantly during all years of grassland use. On average, the increase in dry matter yield obtained over the five years was 0.86 t ha^{-1} (+22%), and the yields in different years varied from 0.70 t ha^{-1} (+14%) to 1.50 t ha^{-1} (+33%). The average dry matter yield of festulolium at nitrogen fertilizer norm of 60 kg ha^{-1} N, was 6.91 t ha^{-1} (from 5.70 t ha^{-1} to 7.86 t ha^{-1}), with further increase in yield to 8.54 t ha^{-1} (from 8.11 t ha^{-1} to 8.96 t ha^{-1}) at 180 kg ha^{-1} N (Fig. 3).

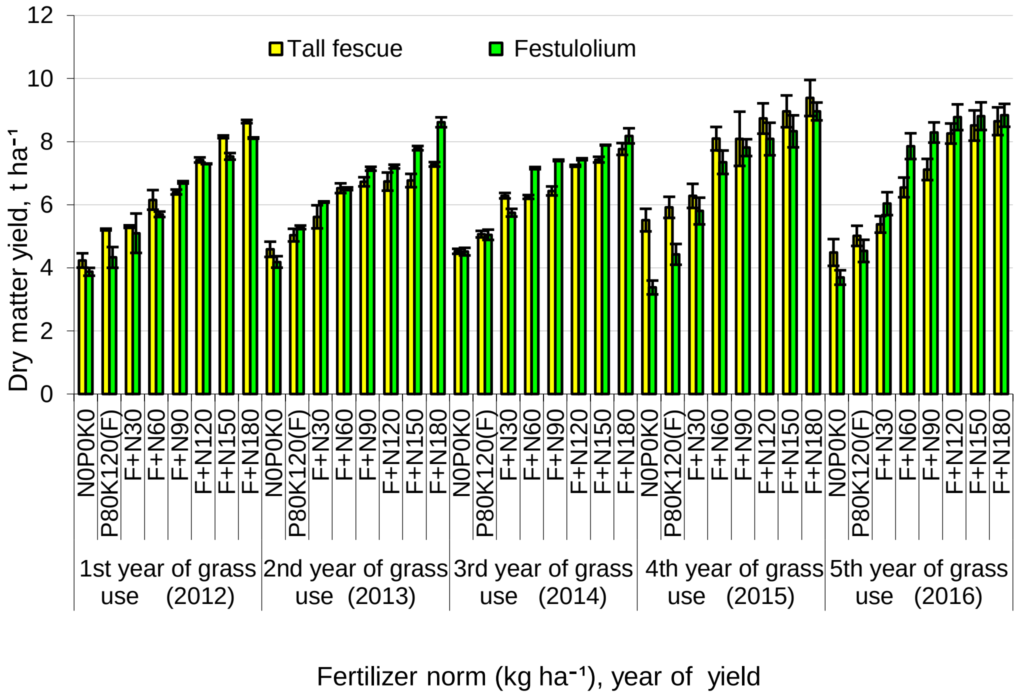


Figure 3. The effect of nitrogen fertilizer on the yield of tall fescue and festulolium.

In assessing the effect of nitrogen fertilizer on the production of high yields, tall fescue and festulolium revealed that significant increases in dry matter yields varied between the years of use. Agrometeorological conditions in the growing year had a significant ($p < 0.001$) effect on tall fescue and festulolium.

In the first year of nitrogen fertilizer use, a significant increase in dry matter yield of tall fescue ($p < 0.05$) was observed at the nitrogen norm of 60 kg ha^{-1} N, when the dry matter yield reached 6.15 t ha^{-1}, and a further increase in nitrogen norm contributed to a significant increase in dry matter yields up to the nitrogen norm of 180 kg ha^{-1} N, when the dry matter yield reached 8.64 t ha^{-1}. Similarly, the dry matter yield ($p < 0.05$) of festulolium increased with the increase in nitrogen fertilizer norm, reaching 8.11 t ha^{-1} of dry matter at 180 kg ha^{-1} N. In the following years, the development of a denser sward of festulolium resulted in the dry matter yield of more than 8.0 t ha^{-1} by applying lower nitrogen rates than those required for the first year of use.

In the second year of tall fescue use, a significant increase ($p < 0.05$) in dry matter yield was achieved at the nitrogen norm of 30 kg ha^{-1} N, and the increase in nitrogen norm continued to provide a significant yield increase of up to 60 kg ha^{-1} N; however, a further increase in nitrogen norm did not result in significant increase in dry matter yield. Also, in the third year of tall fescue use, a significant increase in dry matter yield was gained at the nitrogen norms of 30, 120, and 180 kg ha^{-1} N. In the fourth year of grassland use, a significant yield increase ($p < 0.05$) was detected at the nitrogen norms of 60, 120, and 180 kg ha^{-1} N. In the fifth year of grassland use, a significant increase in dry matter yield was obtained applying nitrogen norms of 60 and 120 kg ha^{-1} N.

From the agronomic point of view, in the first year of tall fescue use, it is necessary to use high nitrogen norms (> 150 kg ha^{-1} N) to exceed the 8.0 t ha^{-1} yield of dry matter. With the age of grassland, when tall fescue becomes stronger and denser, in order to produce a dry matter yield of over 8.0 t ha^{-1}, it is necessary to use 120 kg ha^{-1} N and more.

The linear distribution of polynomials was determined to study the effects of factors such as the year of use, the effect of increasing the nitrogen fertilizer rates, and the importance of increasing nitrogen fertilizer rates. By using linear regression coefficients (Fig. 4), it was found that in the first year of tall fescue use, the highest nitrogen efficiency was achieved with the nitrogen norm of 180 kg ha^{-1} N, in the second and fourth year – with the nitrogen norm of 60 kg ha^{-1} N, in the third year – with the nitrogen norm of 30 kg ha^{-1} N, and in the fifth year – with the nitrogen norm of 120 kg ha^{-1} N.

The estimation of linear coefficients confirmed variations in the significance of the previously established and described growth of dry matter yields. In its turn, the highest efficiency of increasing nitrogen norms in the first year of festulolium use was achieved with the nitrogen norm of 120 kg ha^{-1} N, in the second year – with 30 kg ha^{-1} N, and from the third to fifth year of use – with 60 kg ha^{-1} N.

Linear regression coefficients show that for high yielding of tall fescue (1st year of use) and older grasses (5th year of use), high nitrogen norms (180 kg ha^{-1} N) should be applied (Figs 5 and 6). From 1 kg of N, a dry matter yield of 0.0205 t is produced. In the second and fourth year of tall fescue use, the highest dry matter yields – 0.0248 and 0.0363 t of 1 kg N respectively – were provided by 60 kg ha^{-1} N nitrogen fertilizer, while in the third year of use, the nitrogen fertilizer norm was 30 kg ha^{-1} N, i.e., 0.0407 t of dry matter yield were obtained from 1 kg N. In the second, third and fourth year of tall fescue use, when the highest efficiency of nitrogen fertilizer was detected with nitrogen norms of 30 and 60 kg ha^{-1} N, the use of elevated norms was less effective. The yield of tall fescue in the second year of its use from 1 kg N decreased by 80%, in the third year – by 73%, and in the fourth year – by 68%.

Festulolium

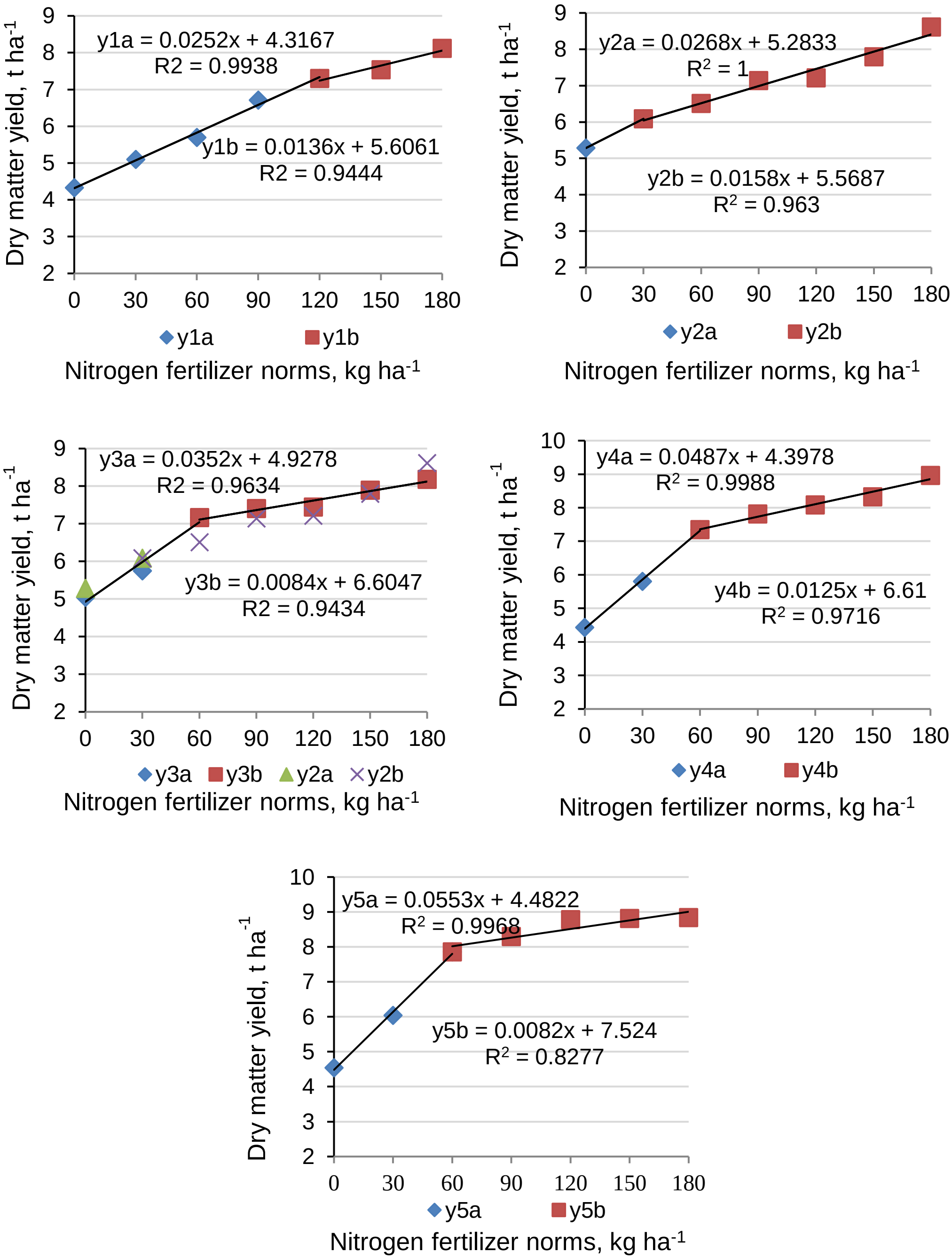
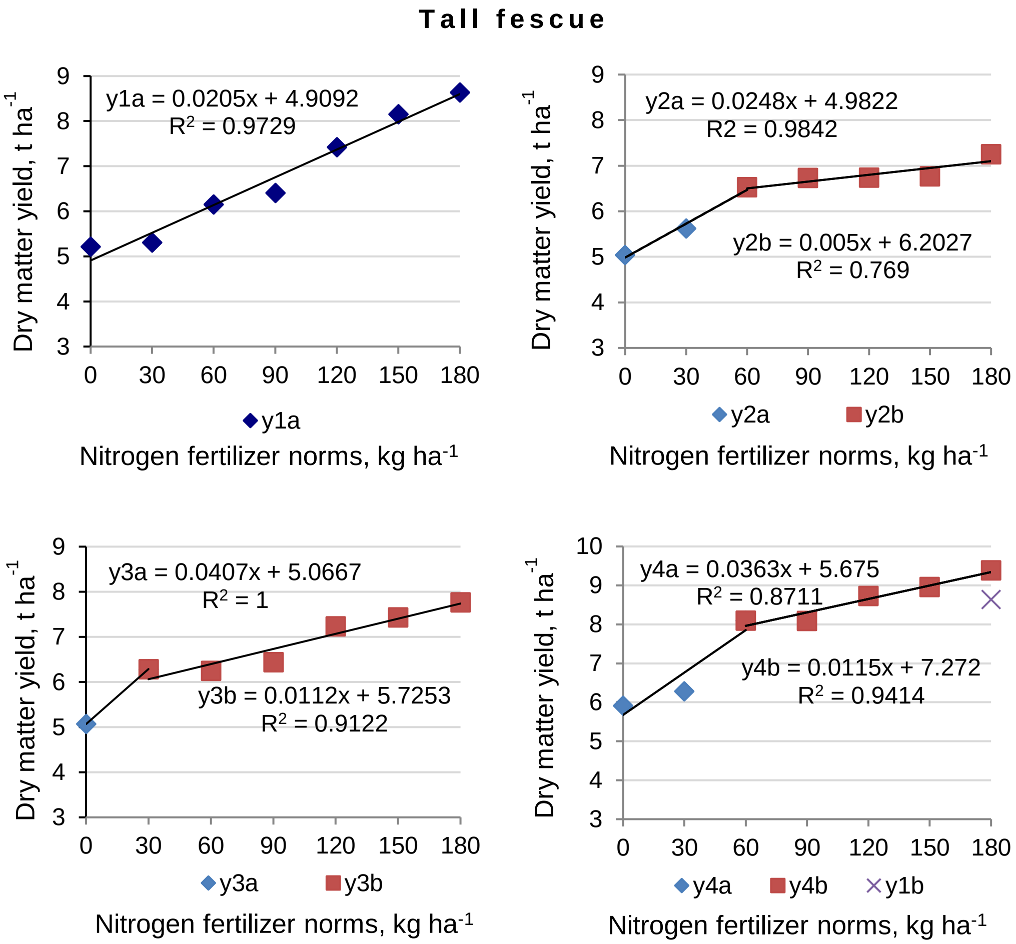
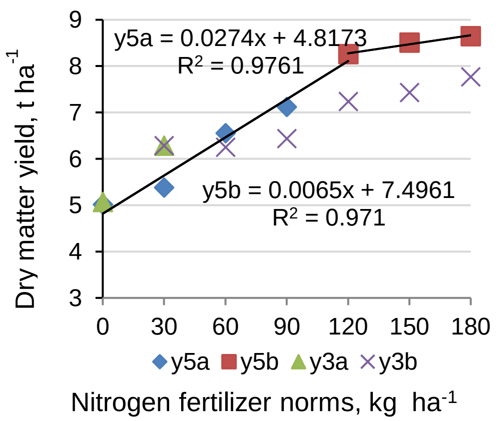


Figure 4 (Continues)





The change in linear regression coefficients ayx un byx definition regions: y1a; y1b – 1st year of use, y2a; y2b – 2nd year of use, y3a; y3b – 3rd year of use, y4a; y4b – 4th year of use, y5a; y5b – 5th year of use.

Figure 4. The efficiency of increasing nitrogen fertilizer norms in the years of festulolium and tall fescue use.

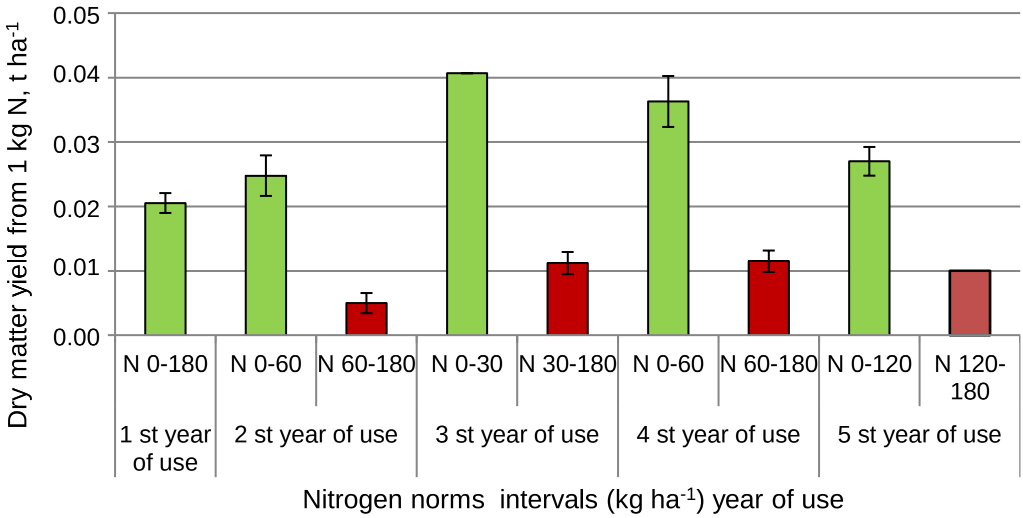


Figure 5. Nitrogen fertilizer efficiency depending on the intervals of nitrogen fertilizer norms for tall fescue.

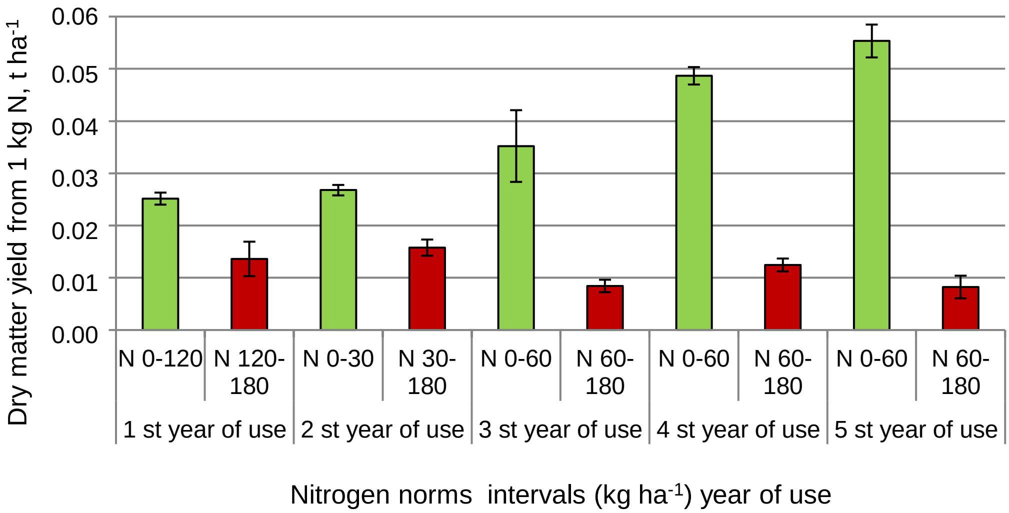


Figure 6. Nitrogen fertilizer efficiency depending on the intervals of nitrogen fertilizer norms for festulolium.

Throughout all years of the experiment, the dry matter yield of festulolium increased concurrently with nitrogen fertilizer norm: in the first year of use, 120–180 kg ha^{-1} N provided the highest fertilizer efficiency; in the second year – accordingly 30–180 kg ha^{-1} N; and from the third to fifth year of use, the most efficient norm was 60–180 kg ha^{-1} N. However, compared to the first two years, nitrogen efficiency decreased. In the first year of use, the reduction was 46%, in the second year – 41%, and in the third, fourth, and fifth year of use, the reduction in dry matter yield was 76%, 74%, and 85%, respectively.

Ash is one of the key indicators characterising the quality of fuel. A too high content of ash and non-combustible minerals (also of sand) causes problems with the automation of combustion process, resulting in the reduction of an effective fuel combustion ratio (Prochnow et al., 2009). Ash content in grass biomass is notably higher than in timber (Volynets & Dahman, 2011; Platače & Adamovičs, 2014).

In this research, ash content in the studied energy grasses was high, reaching on average 7.7%; moreover, nitrogen fertilizers did not have major impact on it. The highest ash content was found in festulolium treated with fertilizer F+30 – 8.6%, and in tall fescue treated with P80K120 (F–background) – 7.9%.

Ash melting temperature has four phases: DT – the initial point of deformation when the sharp peak is rounding; ST – softening temperature, when the ash cone deforms to such extent that the height of the structure reduces to the size of its diameter; HT – the point of the formation of hemisphere, or the cone collapses and becomes dome-shaped; FT – flow temperature, when the liquid ash dissipates along the surface (Kakitis et al., 2009).

At all four ash melting phases, the birch biomass pellets had the temperature above 1,400 °C, but the temperature of grass biomass pellets varied within 1,020–1,200 °C. When burning grass biomass pellets with low ash melting temperature (< 1,000 °C), special attention should be paid to a correct combustion regime. The presence of alkali metals, phosphorus, silicon and calcium is a key determinant of ash melting temperature (Magasiner et al., 2002; Maciejewska et al., 2006); therefore, grass (tall fescue, festulolium) biomass ash melting temperature is lower (1,020–1,270 °C) at all phases, whereas birch biomass temperature is higher and reaches 1,460–1,500 °C.

From the grasses included in the study, the highest calorific value of the first-cut biomass varied from 15.20 MJ kg^{-1} for festulolium to 16.90 MJ kg^{-1} for tall fescue (Table 1).

Table 1. Gross calorific value (Q) for different grass species, MJ kg^{-1}

Reed canary grass	Festulolium	Tall fescue	Average	Sx
16.40	16.50	15.20	15.85	± 0.32

Nitrogen fertilizers contributed not only to the increase in dry matter yield but also to the amount of energy produced. On average, the amount of energy from 1 ha of all nitrogen standard variants varied from 103.9 GJ ha^{-1} for tall fescue to 126.4 GJ ha^{-1} for festulolium. The largest amount of energy received from 1 ha was detected in the variant with a nitrogen norm of 180 kg ha^{-1} N: from 142.6 GJ ha^{-1} for tall fescue to 147.8 GJ ha^{-1} for festulolium.

CONCLUSIONS

1. The grass species and nitrogen fertilization significantly affected the biomass yield of both grasses. The highest dry matter yields of tall fescue (8.96 t ha^{-1}) and festulolium (8.32 t ha^{-1}) were produced when nitrogen fertilizer was applied. Dry matter yields from nitrogen fertilizer levels increased from 10% to 59% for tall fescue, and from 22% to 81% for festulolium.

2. Linear regression coefficients of the polynomial distribution showed that the highest efficiency of nitrogen fertilizer varied over the years of the use of the grasses. The highest nitrogen efficiency for tall fescue was achieved with the norm of 30 kg ha^{-1} N in the 3rd year of use, with the norm of 60 kg ha^{-1} N in the 2nd and 4th year of use, with

the norm of 120 kg ha^{-1} N in the 5th year of use, and with the norm of 180 kg ha^{-1} N in the 1st year of use; whereas for festulolium, the highest nitrogen efficiency was achieved with the norm of 30 kg ha^{-1} N in the 2nd year of use, with the norm of 60 kg ha^{-1} N in the 3rd to 5th year of use, and with the norm of 120 kg ha^{-1} N in the 1st year of use.

3. At all four ash melting phases, the burning temperature of grass biomass pellets varied within 1,020–1,200 °C.

4. From the first cut, the average calorific value of grass biomass was 15.85 MJ kg^{-1}, and the amount of energy produced was 145.29 GJ ha^{-1}.

REFERENCES

Adamovics, A., Gutmane, I. & Adamovica, O. 2017a. The influence of nitrogen fertilisation on the yields and quality of multicomponent sown meadows. *Grassland Science in Europe* **22**, 289–291.

Adamovics, A., Platace, R., Gulbe, I. & Ivanovs, S. 2017b. Influence of fertilizers on chemical content of energy grass biomass. *16th International scientific conference "Engineering for rural development*": proceedings, Latvia, Latvia University of Agriculture, Faculty of Engineering, Latvian Academy of Agricultural and Forestry Sciences. Jelgava, 2017. Vol. **16**, pp. 98–102.

Heinsoo, K., Hein, K., Melts, I., Holm, B. & Ivask, M. 2011. Reed canary grass yield and fuel quality in Estonian farmers' fields. *Biomass and Bioenergy* **35**, 617–625. http://dx.doi.org/10.1016/j.biombioe.2010.10.022

Kaķītis, A., Šmits, M. & Belicka, I. 2009. Suitability of crop varieties for energy production. In: *Engineering for Rural Development. Proceedings of the 8th International Scientific Conference,* May 28–29, 2009. Jelgava, pp. 188–193.

Landström, S., Lomakka, L. & Andersson, L. 1996. Harvest in spring improves yield and quality of reed canary grass as bioenergy crop. *Biomass and Bioenergy* **11**, 333–341.

Lemus, R., Brummer, E.C., Burras, C.L., Moore, K.J., Barker, M.F. & Molstad, N.E. 2008. Effects of nitrogen fertilization on biomass yield and quality in large fields of established switchgrass in southern Iowa, USA. *Biomass and Bioenergy* **32**, 1187–1194.

Lewandowski, I. & Kicherer, A. 1997. Combustion quality of biomass: practical relevance and experiments to modify the biomass quality of *Miscanthus x giganteus*. *European Journal of Agronomy* **6**, pp 163–177.

Lewandowski, I., Scurlock, J.M.O., Lindvall, E. & Christou, M. 2003. The development and current status of perennial rhizomatous grasses as energy crops in the US and Europe. *Biomass and Bioenergy* **25**, 335–361. http://dx.doi.org/10.1016/S0961-9534(03)00030-8

Maciejewska & A., Veringa & H., Sanders & J. & Peteves & S.D. 2006. Co-firing of biomass with coal: constraints and role of biomass pre-treatment. Luxemburg: Office for Official Publications of the European Communities, 100 pp.

Magasiner, N., van Alphen, M., Inkson, M. & Misplon, B. 2002. Characterising Fuels for Biomass – Coal Fired Cogeneration. *International Sugar Journal* **104**(1242), 251–267.

Mulkey, V.R., Owens, V.N. & Lee, D.K. 2008. Management of warm-season grass mixtures for biomass production in South Dakota, USA. *Bioresource Technology* **99**, 609–617.

Nilsson, D., Rosenqvist, H. & Bernesson, S. 2015. Profitability of the production of energy grasses on marginal agricultural land in Sweden. *Biomass and Bioenergy* **83**, 159–168. http://dx.doi.org/10.1016/j.biombioe.2015.09.007

Platače, R. & Adamovičs, A. 2014. The evaluation of ash content in grass biomass used for energy production. *WIT Transactions on Ecology and the Environment* **190**(2), 1057–1065.

Prochnow, A., Heiermann, M., Plöchl, M., Amon, T. & Hobbs, P.J. 2009. Bioenergy from permanent grassland – A review: 2. Combustion. *Bioresource Technology* **100**, 4945–4954.

Scholz, V. & Ellerbrock, R. 2002. The growth productivity and environmental impact of the cultivation of energy crops on sandy soils in Germany. *Biomass and Bioenergy* **23**, 81–92.

Tara, W. Hudiburg, WeiWei, Wang, Madhu, Khanna, Stephen, P. Long,, Puneet Dwivedi, William, J. Parton, Melannie, Hartman & Evan, H. DeLucia. 2016. Impacts of a 32-billion-gallon bioenergy landscape on land and fossil fuel use in the US. *Nature Energy* **1**, doi:10.1038/nenergy.2015.5

Tilvikiene, V., Kadziuliene, Z., Dabkevicius, Z., Venslauskas, K. & Navickas, K. 2016. Feasibility of tall fescue, cocksfoot and reed canary grass for anaerobic digestion: Analysis of productivity and energy potential. *Industrial Crops and Products* **84**, 87–96. http://dx.doi.org/10.1016/j.indcrop.2016.01.033

Tonn, B., Thumm, U. & Claupein, W. 2010. Semi-natural grassland biomass for combustion: influence of botanical composition, harvest date and site conditions on fuel composition. *Grass and Forage Science* **65**, 383–397. http://dx.doi.org/10.1111/j.1365-2494.2010.00758.x

Volynets, B. & Dahman, Y. 2011. Assessment of pretreatments and enzymatic hydrolysis of wheat straw as a sugar source for bioprocess industry. *International Journal of Energy and Environment* **2**, 427–446.

Agronomy Research **17**(6), 2158–2171, 2019
https://doi.org/10.15159/AR.19.212

Use of olive pomace as an amendment to improve physico-chemical parameters of soil fertility

H. Ameziane[1,*], A. Nounah[1], M. Khamar[1] and A. Zouahri[2]

[1]Mohammed V University, High School of Technology, Civil Engineering and Environment Laboratory (LGCE), Materials Water and Environment team, MA11060 Sale, Morocco
[2]INRA, Regional Center for Agricultural Research in Rabat, Research unit on Environment and Conservation of Natural Resources, MA10112 Rabat, Morocco
[*]Correspondence: amezianehalima@gmail.com

Abstract. Given their richness in nutritive elements, the majority of agricultural waste is used as soil amendments, including olive oil waste. The purpose of this study is to determine the effects of the use of olive pomace from three extraction systems on the physico-chemical fertility of the soil, after their use as an amendment for faba bean cultivation. The experiment is carried out at the Civil Engineering and Environment Laboratory in the EST of Salé, in pots where the olive pomace has been mixed with the soil, respecting the percentages studied. Several relative physico-chemical parameters of soil fertility were determined at the end of the experiment, namely pH, electrical conductivity (EC), total kjeldhal nitrogen (NTK) content, organic carbon and exchangeable bases concentration and soil cation exchange capacity (CEC) determination. Different percentages of pomace from the three extraction systems were applied (control, 10%, 15%, 25%, 50%, 75% and 100%) for four months of bean germination test. The application of the pomace reduced soil pH, and increased soil organic matter and organic carbon content in proportion to the added percentage of pomace. The available phosphorus and exchangeable potassium content increased significantly ($p < 0.05$) in pots containing different percentages of pomace compared to their concentrations in the soil (control). The total nitrogen content has not increased sufficiently but remains significantly different from the control, especially for the percentages of 25%, 50% and 75%. For its part, the cation exchange capacity (CEC) is important and will allow a good retention of nutrients for all percentages.

Key words: olive pomace, physico-chemical fertility, soil amendment.

INTRODUCTION

The waste management from olive crushing plays a key role in the sustainable production of olive oil and is a major environmental problem, particularly in Mediterranean countries, which produce more than 95% of olive oil worldwide (Roig et al., 2006). The extraction of olive oil can be carried out by several processes, including the three extraction systems mentioned in this study: the traditional pressing system based on manual pressing of olives and natural separation of oil from olive mill wastewater by simple settling, the continuous system based on the use of a centrifuge to ensure the separation of oil from the paste resulting from the crushing of olives. The paste is separated into two phases (olive oil and wet pomace) in the case of a continuous two-phase system and into three phases (olives mill wastewater, pomace and olive oil) in the case of the continuous three-phase system Fig. 1.

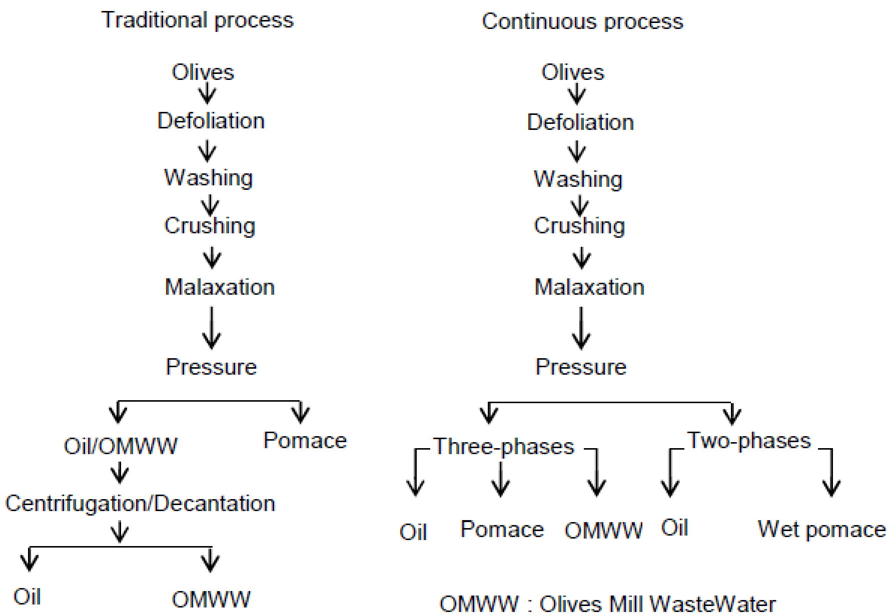


Figure 1. Extraction processes of olive oil.

Pomace and olive mill wastewater together represent potential threats to the environment due to the large amount produced over a short period of time on the one hand, and their high organic load on the other (Morillo et al., 2009). Several recovery methods have been proposed to reduce the residual impact of this waste on the environment. Indeed, the pomace has been used as fuel, adsorbent, livestock feed, thermal insulation and fertilizer (Federici et al., 2009; Morillo et al., 2009).

Direct application of pomace to agricultural land appears to be operationally simple and economically achievable. In addition, in some European countries, national legislation allows the application of specific quantities of pomace on cultivated land. In Italy for example, the spreading of olive pomace on agricultural land can be done in accordance with Law No. 574 of 1996.

The application of raw or composted pomace on agricultural land has been the subject of several in-depth studies (Di Serio et al., 2008; Mechri et al., 2009; Chartzoulakis et al., 2010).

As a result, the olive pomace can be considered a good fertilizer because of its high levels of nutrients needed for plant growth and development, namely potassium (K), nitrogen (N), phosphorus (P), magnesium (Mg), and organic matter (Niaounakis et al., 2006; Lozano-García et al., 2011). In addition, the incorporation of these wastes into the soil is an interesting contribution to carbon sequestration, the reduction of runoff and soil loss through erosion (Mondini & Sequi, 2008; Lozano-García et al., 2011), without negatively affecting the soil microbial community (Proietti et al., 2015), which plays a significant role in the assimilation of certain nutritive elements necessary for plants.

The olive pomace use as a soil amendment has given satisfactory results in terms of yield and crop production (Lopez-Pineiro et al., 2006) due to its high content of macro and microelements (Ameziane et al., 2018). Therefore, the study of the effect of pomace on the physico–chemical fertility of the soil is necessary to ensure sustainable olive growing that respects the environment without negative effects on the soil. The objective of this work is to study the effect of the use of olive pomace at different percentages as soil amendment on the physico-chemical characteristics of Moroccan sandy soil, in order to improve the fertility parameters of these soils, for a better agricultural exploitation of the latter.

MATERIALS AND METHODS

Materials

The olive pomace was collected from three oil mills in the city of Tahla (Tahla, Morocco, latitude 34°02'58" North, longitude 4°25'17" West, altitude above sea level: 606 m). The first oil mill operates on a two–phase continuous system, the second on a three-phase continuous system and the third ensures a traditional crushing. The present olive pomace was used as fertilizer at different percentages (0%, 10%, 15%, 25%, 50%, 75%, 100%) in pots for *faba bean* cultivation, to see the influence of the use of this pomace on the physico-chemical fertility of the soil. The test began with drying the pomace, grinding and sieving it to 1 mm, then seeding the bean grains in pots with different percentages of soil and pomace from the three extraction systems, averaging 5 grains per pot. The test lasted four months from February 2017 to May 2017 at the civil engineering and environment laboratory of the high school of technology in Salé-Marocco, at a variable temperature and humidity depending on outdoor climatic conditions.

Each pot was regularly irrigated three times a week with an equal amount of well water. The test was repeated three times.

The physico–chemical characteristics of the pomace studied are presented in Table 1 (Ameziane et al., 2018).

The soil used in this study comes from 'Salé' (Salé, Morocco, latitude 34°03'11" North, longitude 6°47'54" West, altitude above sea level: 34 m). It was sampled from the topsoil (0–20 cm) of a callistemon. It is a sandy soil and its properties are presented in Table 1 (Ameziane et al., 2018).

At the end of the plant period, the soil contained in the pots is dried at 40°C and stored for physico–chemical analysis.

Table 1. Physico-chemical parameters of the pomace from the three extraction systems and the soil (Ameziane et al., 2018)

Physico-chemical parameters	Olive pomace			Soil
	Olive pomace from the traditional press system (GT)	Olive pomace from the two-phase system (G2Ph)	Olive pomace from the three-phase system (G3Ph)	
Clay in %	-	-	-	9 ± 0.044
Sand in %	-	-	-	68 ± 0.088
Silt in %	-	-	-	23 ± 0.044
pH	6.04 ± 0.02	5.90 ± 0.02	5.94 ± 0.03	7.61 ± 0.044
Electrical conductivity in mS cm^{-1}	2.62 ± 0.10	2,47 ± 0.09	3.21 ± 0.36	0.085 ± 0.149
Moisture in %	43.33 ± 1.87	52.81 ± 0.77	53.56 ±1.63	4.51 ± 0.044
Organic matter in %	77.02 ± 0.88	74.69 ± 13.36	92.03 ± 4.65	1.58 ± 0.158
Total organic carbon in %	49.72 ± 0.36	49.32 ± 0.36	50.79 ± 0.31	0.83 ± 0.597
Total nitrogen in %	0.00257	0.00175	0.00402	0.134
The available Phosphorus in %	0.00015	0.02	0.04	0.0103
Calcium in %	0.29	0.23	0.28	0.48
Exchangeable potassium in %	6.70	4.60	9.4	0.0445
Magnesium in %	0.04	0.03	0.03	0.0243
Sodium in %	0.9	1	1	0.0092

The values obtained represent the average of three repetitions.

The water used for faba bean irrigation comes from a well of the Higher School of Technology of Sale, whose physico–chemical characteristics (Table 2) are in compliance with the Moroccan standard for irrigation water (Secretariat of State at the Ministry of Energy, Mines, Water and Environment, in charge of Water and Environment, 2007):

Table 2. Physico-chemical parameters of irrigation water (Ameziane et al., 2018)

Physico–chemical parameters	Characteristics of irrigation water
pH	7.49 ± 0.047
Electrical conductivity in mS cm^{-1}	1.024 ± 0.016
Temperature in °C	18.13 ± 0.058
Suspended matter mg L^{-1}	0.232 ± 0.033
Salinity in ppb	655 ± 0.1
Nitrates in mg L^{-1}	7.04 ± 0.007
Chlorides in mg L^{-1}	192.5 ± 0.044
Boron in mg L^{-1}	0
Sulphates in mg L^{-1}	21.33 ± 0.005
Ortophosphates in mg L^{-1}	0.0134 ± 0.0001
The sodium absorption ratio (SAR) $meq^{1/2}$ $(l^{1/2})^{-1}$	0.53 0.5

The values obtained represent the average of three repetitions.

Methods

To carry out physico-chemical characterization, a representative soil sample is sieved to 2 mm and dried in an oven at 40 °C to determine the pH, electrical conductivity (EC), total organic carbon (TOC), exchangeable potassium (K_2O), available phosphorus

(P_2O_5), total Kjeldhal nitrogen (NTK), exchangeable sodium (Na^+), cation exchange capacity (CEC).

pH and EC are measured in the aqueous extract using the ratio 2:5 (w/v) for pH (Rodier et al., 2009), and the ratio 1:5 (w/v) for EC (ISO 11265, 1994).

Total nitrogen is measured by the Kjeldahl method (Bremner et al., 1982), total organic carbon by the Walkley and Black method (Walkley & Black, 1934) and organic matter was estimated by the following formula OM in % = %TOC×1.72 (Dabin, 1970).

Available Phosphorus by the Olsen method (Olsen, 1954). Exchangeable potassium and sodium are extracted by CH_3COONH_4 at pH 7 (Bower et al., 1952) and measured by flame photometer. The cation exchange capacity (CEC) was obtained by the Mehlich method (1942). The physico-chemical analyses were carried out in the environment and natural resources conservation laboratory of the Agricultural Research National Institute in Rabat.

Statistical analyzes

The obtained results correspond to the average of 3 repetitions. The experimental data were subjected to unidirectional variance analysis (ANOVA) and mean separations were made by the smallest difference (LSD) at the significance level of $p < 0.05$, using the Statgraphics centurion XVI program for Windows.

RESULTS AND DISCUSSION

Chemical characteristics of the soil and olive pomace

In this study, the used soil is sandy (68%) with a neutral pH (7.61) and an average moisture of 4.51%. It has a low content of organic matter (1.58%) as well as organic carbon (0.83%), which is the case for most Mediterranean soils (Ganlanakis, 2017). Its content of total nitrogen and available phosphorus is high and is worth respectively (0.134%) (0.0103%) as well as potassium (0.0445%) (Table 1).

The pomace average humidity varies from 43.33% to 53.56% depending on the used extraction system. They have a slightly acidic pH (5.9 to 6.04) and a very high content of organic matter (74.69% to 92.03%) and organic carbon (49.32 to 50.79%). They are low in nitrogen (0.00175% to 0.00402%), phosphorus (0.00015% to 0.04%) and sodium (0.9% to 1%) but rich in potassium (4.6% to 9.4%) (Table 1).

Olive pomace effect on the physical characteristics of the soil (pH, EC, OM)

pH

The different soil types pH varied slightly from basicity to neutrality during the germination test. Indeed, more the concentration of pomace increases, more the pH turns towards neutrality (Fig. 2). On the other hand, this pH remains favourable for the assimilation of mineral salts and micro-nutrients essential for the growth and development of most plants (Nefzaoui, 1985). Despite the acidity of the pomace (Table 1), the germination of the faba bean increased the pH to neutral even for the pot containing 100% pomace. This can be attributed to the microbiological activity of the microorganisms. Actually, when the soil is enriched with easily decomposable organic compounds, the release of ammonia always leads to a more or less durable increase in pH, depending on the doses and the plants' use (Moureaux, 1973).

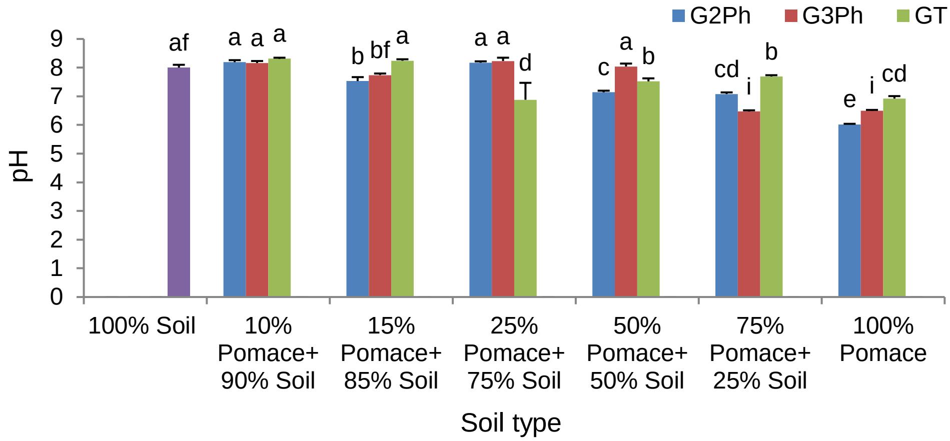


Figure 2. Effect of pomace addition on soil pH (Values with different letters are significantly different: $p < 0.05$).

Electrical conductivity

The electrical conductivity (EC) varies according to the added pomace quantity (Fig. 3), in fact the higher the pomace percentage is, the higher is the conductivity. This is normal given the richness of pomace in soluble salts, especially potassium salt (Table 1). However, the optimal tolerance range for the electrical conductivity of soils, which depends on the species and phase of cultivation, should not reach 2.5 mS cm^{-1} in order to avoid reverse osmosis and root drying (Montoroi, 1996). It can be said that the contribution of olive pomace to the soil studied did not significantly affect soil salinity, however there is a variation in soil salinity depending on the used type of pomace.

The pomace electrical conductivity variation is significantly different between the three extraction systems pomace. This can be attributed to the variation in the extraction process, the way olives are collected and preserved before extraction (Chimi, 2001).

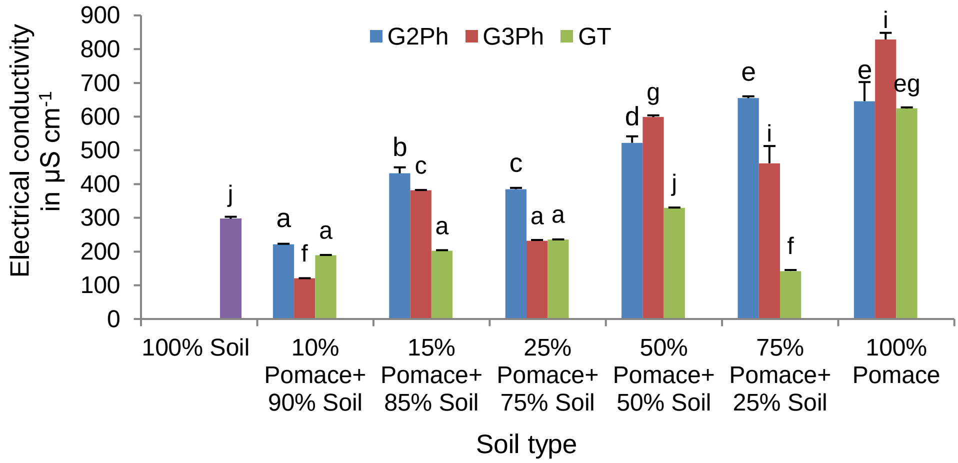


Figure 3. Effect of pomace addition on soil EC (Values with different letters are significantly different: $p < 0.05$).

Organic matter

Given the high organic matter of olive pomace, its addition to the soil has increased the concentration of the latter. This increase is significantly different between the different percentages of pomace for the three extraction systems (Fig. 4). The high content of organic matter in the soil promotes water retention and increases the overall stability of the soil (Lopez-Pineiro et al., 2007), which makes soils less susceptible to erosion. A similar study conducted in Turkey by Kavdir & Kili (2007) showed that the distribution of pomace on the soil improves its structure, stability and increases water retention capacity.

According to Fig. 4, the results obtained are similar for the pomace from the thre extraction systems.

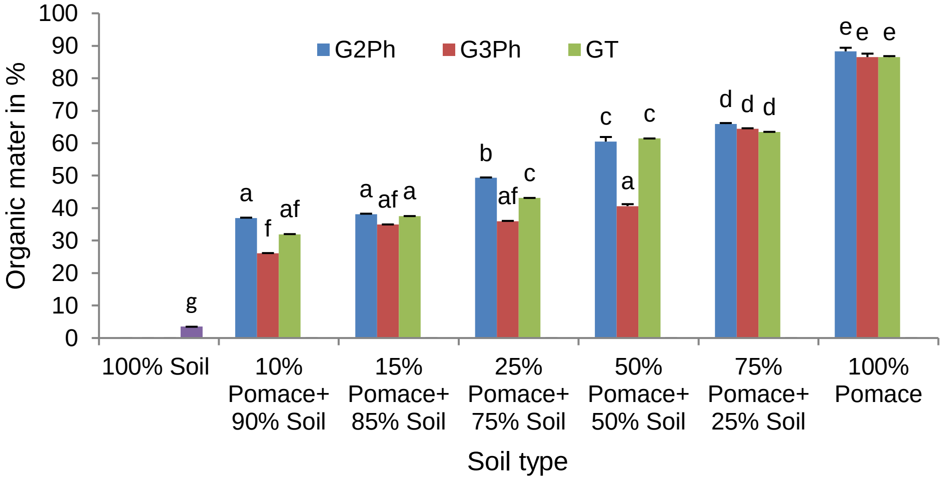


Figure 4. Effect of pomace addition on organic matter content (Values with different letters are significantly different: $p < 0.05$).

Effect on soil chemical parameters

Total organic carbon

Following the contribution of pomace to the soil in terms of organic matter, the organic carbon concentration will also be high. Indeed, there is a significantly different increase in the organic carbon concentration depending on the added pomace concentration (Fig. 5). In addition, the experiment conducted in the short and medium term by López-Piñeiro et al. (2008) in Spain showed a significant increase in the amount of organic carbon in soil treated for three years with a pomace from the two-phase system. This result is consistent with those obtained by other authors (Madejon et al., 2003; Montemurro et al., 2004), but it is necessary to determine the quantity and quality of the added pomace, as well as their application techniques. In fact, organic matter mineralization is enhanced when the pomace is distributed on the soil surface without incorporation (Proietti et al., 2015).

In terms of comparison between the pomace from the three extraction systems, it is clear that the pomace from the two-phase system provides a significant amount of TOC to the soil. Indeed, the TOC content for all percentages for this pomace type is significantly different from the control.

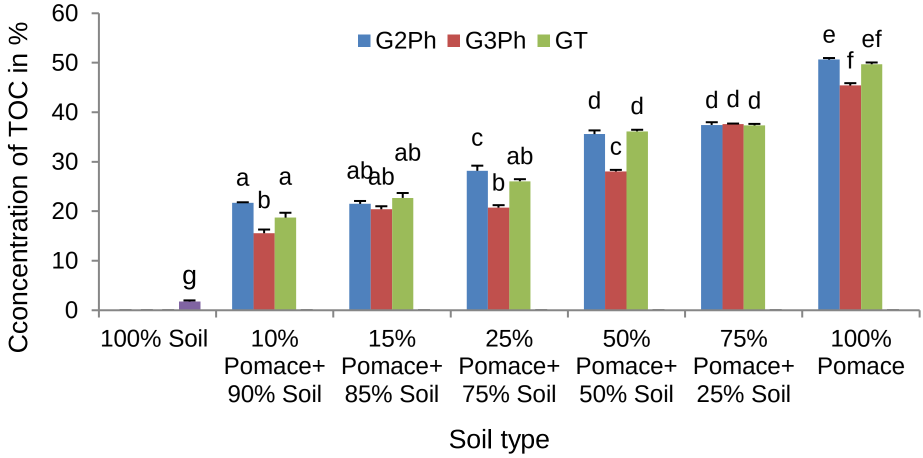


Figure 5. Effect of pomace addition on TOC content in soil (Values with different letters are significantly different: $p < 0.05$).

Total nitrogen

The initial soil (control) nitrogen concentration is 0.134% (Table 1), but following the faba bean germination test the plant assimilates its nitrogen needs and consequently the nitrogen concentration has decreased to 0.04% (Fig. 6). However, due to the addition of olive pomace, the concentration has increased, especially in pots containing 25%, 50%, 75% and 100% pomace mainly for those coming from the continuous system.

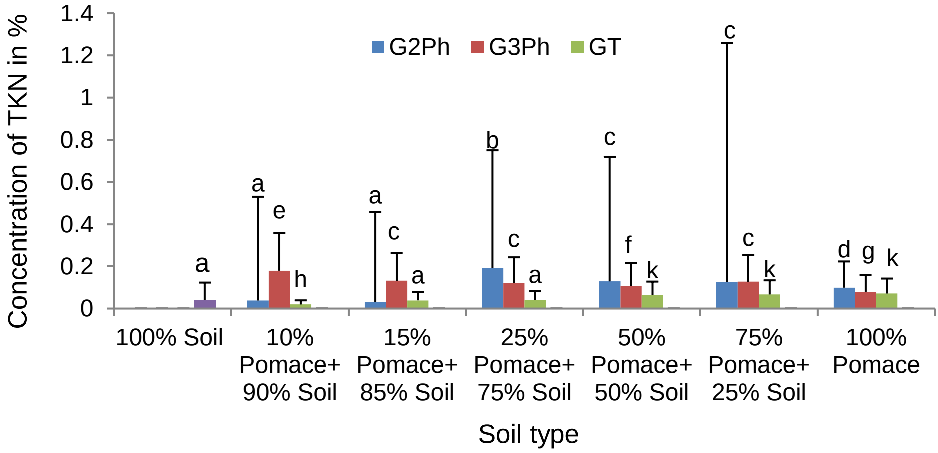


Figure 6. Effect of pomace addition on NTK content in soil (Values with different letters are significantly different: $p < 0.05$).

The same results were observed in an Italian study. Brunetti et al. (2005) showed that the application of olive pomace for two years, allowed a linear increase in total soil nitrogen in the first year, attributed to the increase in organic nitrogen content, but in the second year it is due to the increase in inorganic nitrogen. Despite the increase in nitrogen obtained by comparison with the control, olive pomace does not really have a sufficient nitrogen supply in comparison with the plants' needs. Indeed, when the nitrogen concentration is less than 1.5%, the soil immobilizes the nitrogen, while at a concentration higher than 2% the mineral nitrogen is unconfined (MacNaeidhe & Courtney, 1983).

Available phosphorus

When an amendment is applied to the soil, an increase in the available phosphorus is expected, as the main result of improved chemical fertility.

Given the low phosphorus concentration in the studied pomace (Table 1), the addition of the latter did not significantly increase the phosphorus concentration in the majority of percentages (Fig. 7). The same result has been obtained by other authors, indeed Nasini et al. (2013) and Brunetti et al (2005) have shown that the application of different types of olive pomace does not affect the available phosphorus of the soil.

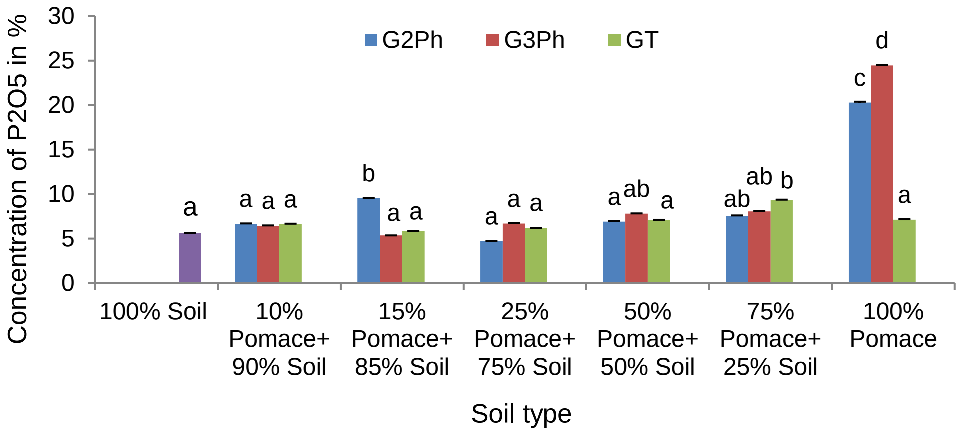


Figure 7. Effect of pomace addition on P_2O_5 content in soil (Values with different letters are significantly different: $p < 0.05$).

In addition, significant increases in soil available phosphorus have been found when pomace (rich in phosphorus) are used as soil amendment (Madejon et al., 2003; Paredes et al., 2005; López-Piñeiro et al., 2008). Indeed, the increase in available phosphorus is the consequence of the phospho-humic complex formation, which prevents the phosphorus insolubilisation in acid and calcareous environments (Giusquiani et al., 1995). Also a study conducted in Italy by Proietti et al (2015) showed that phosphorus increase occurs only in the first 15 cm of treated soil, while no difference was observed in the 15–30 cm layer between the control soil and the treated soil.

The pomace phosphorus addition to the soil is identical for the pomace from the three extraction systems.

Exchangeable potassium

The studied pomace have significant levels of exchangeable potassium in comparison to soil (Table 1). As a result, the higher is the percentage of pomace, the higher is the potassium concentration in the soil. However, this increase remains significant for pomace from the continuous system (Fig. 8). Our results are similar to those of other authors. Indeed, an increase of 4.9 times in exchangeable potassium compared to the control is obtained after 3 years of treatment in an Italian study, when olive pomace is used as an amendment (Proietti et al., 2015). In addition, adequate potassium fertilization allows better tolerance to drought, which is very frequent under Mediterranean conditions (Ganlanakis, 2017).

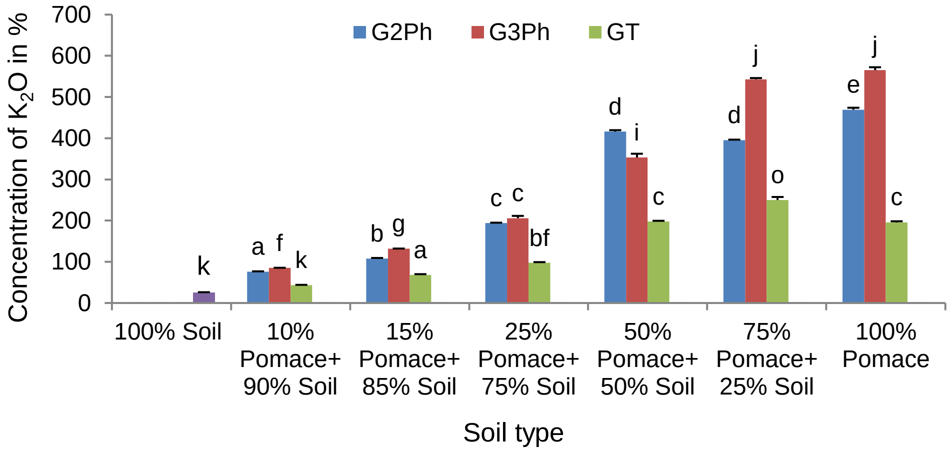


Figure 8. Effect of olive pomace addition on soil K_2O content (Values with different letters are significantly different: $p < 0.05$).

Sodium

The germination of the faba bean allowed an increase in the sodium concntration (Fig. 9) in the different percentages including the control compared to their initial concentration (Table 1).

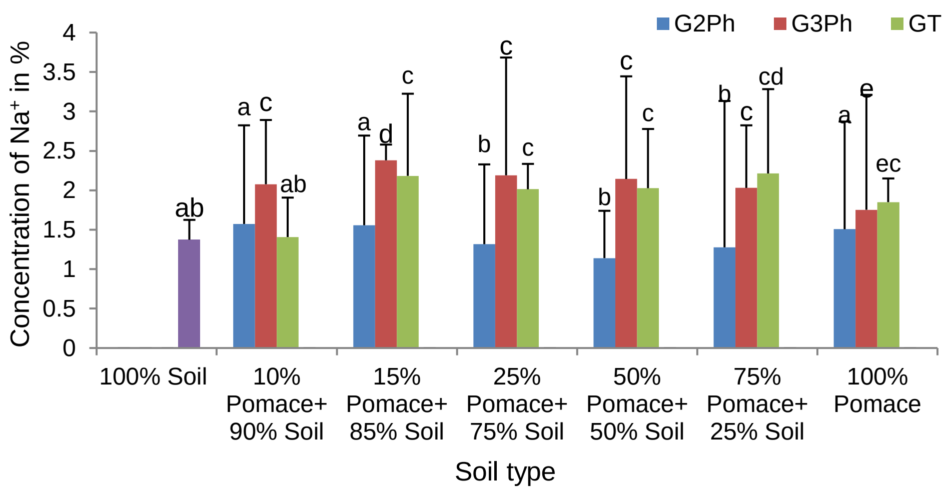


Figure 9. Effect of olive olive pomace addition on Na^+ content in soil (Values with different letters are significantly different: $p < 0.05$).

The sodium concentration for pots containing different percentages of pomace from the three-phase system and the traditional system is significantly high ($p < 0.05$) compared to the control. As a result, the pomace from these two extraction systems has increased the sodium concentration in the soil, and this is due to the high organic matter content and the soil ability to retain cations. Indeed, even if the sodium is the most abundant of all alkaline metals, by a representation of 2.6% of the earth's crust (Weast, 1972), it is retained with a low energy on the CEC, so it is easily washable and quickly replaceable by bivalent cations (Mg^{2+}, Ca^{2+}...) (Dabin, 1970). But with a high organic matter content, the storage reservoir for cationic nutrients increases (Duchaufour, 1954), thus increasing the concentration of these elements in the soil, of which sodium is a part.

Cation exchange capacity (CEC)

The cation exchange capacity of the soil containing different percentages of olive pomace is significantly different compared to the control ($p < 0.05$) (Fig. 10).

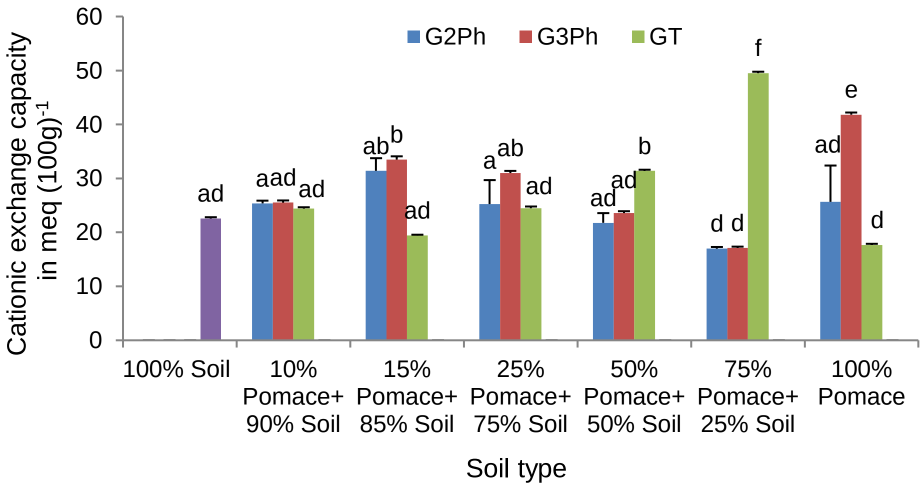


Figure 10. Effect of olive pomace addition on cation exchange capacity of the soil (Values with different letters are significantly different: $p < 0.05$).

It is accepted that the higher the soil CEC, the more it can retain cations, which improves its structure. The soil cation exchange capacity has significantly increased for the different percentages of pomace compared to control. This increase can be attributed to the high concentration of organic matter in pomace. Indeed, CEC is linked to the clay-humic complex, and is therefore dependent on the quantities of clay and OM it contains (Duchaufour, 1954). And given the pomace high organic matter, an increase in cation exchange capacity is expected as the main result of soil amendment by these pomace. As a result, olive pomace has certainly increased the soil reservoir of cations (K^+, Mg^{2+},Ca^{2+},Na^+), and thus fertilization interventions will be less and the plant will always have enough food.

The results are consistent with other studies using olive pomace as a fertilizer (Paredes et al., 2005; Lopez-Pineiro et al., 2006).

The pomace addition to the sandy soil of the High School of Technology of Sale has improved the soil chemical fertility, whatever the extraction system used is. Indeed, the objective was to see which type of olive pomace would provide an optimal contribution to the soil in terms of fertilizing element. While the results obtained were identical for the pomace from the three extraction systems.

All the results obtained during this experiment approve that the use of olive pomace as an amendment has positive effects on the physical and chemical characteristics of the soil. These results are consistent with other conducted studies, in terms of physical characteristics, the diffusion of pomace in the soil increases its porosity, water retention capacity and overall stability (Giusquiani et al., 1995; Kavdir & Killi, 2007). The increase in overall stability is attributed to an increase in organic matter (Lopez-Pineiro et al., 2007), which makes soils less susceptible to erosion.

In terms of chemical characteristics, the pomace addition over time improves the chemical fertility of the soil by increasing organic matter and nutrient content (total nitrogen, phosphorus and exchangeable potassium) without changing the pH value and soil salinity (Montemurro et al., 2004; Cucci et al., 2008; Chartzoulakis et al., 2010). In an experiment conducted in central Italy, the application of large quantities of pomace (50 t ha^{-1}) in an olive grove for 4 consecutive years caused a slight decrease in pH, an increase in organic matter, total nitrogen, exchangeable potassium, magnesium and phosphorus content (Nasini et al., 2013). Generally olive pomace contains a large amount of potassium salts, therefore, an increase in the exchangeable potassium content is expected in pomace-enriched soils (Proietti et al., 2015). On the other hand, adequate potassium fertilization allows a better tolerance to drought, which is very frequent under Mediterranean, conditions (Ganlanakis, 2017).

The proper use of pomace can be considered a valuable method to increase the soil chemical and physical fertility while saving the high costs of chemical fertilizers.

CONCLUSION

The olive pomace application at doses of 10, 15, 25, 50, 75, and 100% modified the soil pH from basicity to neutrality, it increased the electrical conductivity of the soil, as well as the organic matter and organic carbon content compared to the control. The total nitrogen content has increased slightly, but remains insufficient in terms of fertility. For available phosphorus, the concentration is not significantly different for the majority of percentages. The exchangeable potassium is proportional to the percentages of the added pomace. Given the pomace richness in organic matter, the cation exchange capacity has increased for the different percentages, increasing with it the soil reserves in exchangeable bases. As a result, olive pomace has great potential to improve the structure of coarse-textured soils in the short term, in most Moroccan soils.

REFERENCES

Ameziane, H., Nounah, A., Khamar, M., Cherkaoui, E. & Kabbour, MR. 2018. Agrochemical Characterization of Olive Pomace Obtained by Different Systems of Extraction. *Der Pharma Chemica* **10**(12), 33–40.

Bower, C.A., Reitemeier, R.F. & Fireman, M. 1952. Exchangeable cation analysis of saline and alkali soils. *Soil Sci*. **73**, 251–261.

Bremner, J.M. & Mulvaney, C. S. 1982. Nitrogen-Total. In: Methods of soil analysis, part 2. A.L. Page, et al. (eds.). Agronomy Monog. 9 ASA and SSSA, Madison, WI, pp. 595–624.

Brunetti, G., Plaza, C. & Senesi, N. 2005. Olive pomace amendment in Mediterranean conditions: effect on soil and humic acid properties and wheat (Triticum turgidum L.). *J. Agric. Food Chem*. **53**, 6730–6737.

Chartzoulakis, K., Psarras, G., Moutsopoulou, M. & Stefanoudaki, E. 2010. Application of olive mill wastewater to a Cretan olive orchard: effects on soil properties, plant performance and the environment. *Agric. Ecosyst. Environ*. **138**(3–4), 293–298.

Chimi, H., Quality of olive oils in Morocco, 2001. *PNTTA Monthly Bulletin and Liaison*, **79**, pp. 1–4 (in French).

Cucci, G., Lacolla, G. & Caranfa, L. 2008. Improvement of soil properties by application of olive oil waste. *Agron. Sustain. Dev*. **28**, 521–526.

Dabin, B. 1970. *Chemical factors of soil fertility (exchangeable bases; salts; use of fertility scales)* – chapitre XI, pp. 221–237 (in French).

David, R. Lide, ed., CRC. 2005. Handbook of Chemistry and Physics, Internet Version, < http://www.hbcpnetbase.com>,CRC Press, BocaRaton, FL, 2661 pp.

Di Serio, M.G., Lanza, B., Mucciarella, M.R., Russi, F., Iannucci, E., Marfisi, P. & Madeo, A. 2008. Effects of olive mill wastewater spreading on the physico-chemical and microbiological characteristics of soil. *Int. Biodeterior. Biodegradation* **62**(4), 403–407.

Duchaufour Ph. 1954. *Properties of humic complexes in different soil types*. Ecole Nationale eaux et forêts, Nancy, 29 pp. (in French).

Federici, F, Fava, F, Kalogerakis, N & Mantzavinos, D. 2009. Valorisation of agro-industrial by products, effluents and waste: concept, opportunities and the case of olive mill wastewaters. *Chem Technol Biotechnol* **84**, 895–900.

Fendorf, S.E. 1995. Surface reactions of chromium in soils and waters. *Geoderma* **67**, 55–71.

Ganlanakis Charis, M. 2017. *Olive Mill Waste. Recent Advances for Sustainable Management. Galanakis* Laboratories, Chania, Greece : Academic press, 300 pp.

Giusquiani, P.L., Pagliai, M., Gigliotti, G., Businelli, D. & Benetti, A. 1995. Urban waste compost: effects on physical, chemical and biochemical soil properties. *J. Environ. Qual*. **24**, 175–182.

ISO 11265. Soil quality - Determination of the specific electrical conductivity. October 1994 (in French).

Kavdir, Y. & Killi, D., 2007. Influence of olive oil solid waste applications on soil pH, electrical conductivity, soil nitrogen transformations, carbon content and aggregate stability. *Bioresour. Technol*. **99**, 2326–2332.

Law n. 574. 11th November 1996. New standards for the agronomic use of vegetation water and oil mill discharges. Published in the Official Gazette n. 265 del 12 novembre 1996 (in Italian).

Lopez-Pineiro, A., Fernandez, J., Nunes, R., Manuel, J. & Garcia Navarro, A. 2006. Response of soil and wheat crop to the application of two-phase olive mill waste to Mediterranean agricultural soils. *Soil Sci*. **171**, 728–736.

Lopez-Pineiro, A., Murillo, S., Barreto, C., Munoz, A., Rato, J.M., Albarran, A. & Garcia, A. 2007. Changes inorganic matter and residual effect of amendment with two-phase olive-mill waste on degraded agricultural soils. *Sci. Total Environ*. **378**(1–2), 84–89.

Lopez-Pineiro, A., Albarran, A., Rato Nunes, J.M. & Barreto, C. 2008. Short and medium term effects of twophase olive mill waste application on olive grove production and soil properties under semiarid Mediterranean conditions. *Bioresour. Technol*. **99**, 7982–7987.

Lozano-García, B., Parras-Alcántara, L. & del Toro Carrillo de Albornoz, M. 2011. Effects of oil mill wastes on surface soil properties, runoff and soil losses in traditional olive groves in southern Spain. *Catena* **85**, 187–193.

MacNaeidhe, F.S. & Courtney, J. 1983. Vegetable crop production on peat soil. Proceedings *International Symposium on Peat Utilization*, Bemidji State University, Minnesota, U.S.A., pp. 229–238.

Madejon, E., Burgos, P., Lopez, R. & Cabrera, F. 2003. Agricultural use of three organic residues: effect on orange production on properties of a soil of the Comarca Costa de Huelva (SW Spain). *Nutr. Cycl. Agroecosyst*. **65**, 281–288.

Mechri, B., Issaoui, M., Echbili, A., Chehab, H., Mariem, F.B., Braham, M. & Hammami, M. 2009. Olive orchard amended with olive mill wastewater: effects on olive fruit and olive oil quality. *J. Hazard. Mater*. **172**, 1544–1550.

Mehlich, A. 1942. Rapid estimation of base-exchange properties of soils. *Soil Sci.* **53**, 1–14.

Mondini, C. & Sequi, P. 2008. Implication of soil C sequestration on sus-tainable agriculture and environment, *Waste Manage*. **28**, 678–684.

Montemurro, F., Convertini, G. & Ferri, D. 2004. Mill wastewater and olive pomace compost as amendments for rye-grass. *Agronomie* **24**, 481–486.

Montoroi, Jean-Pierre. 1996. *Sustainable mangrove soil management in Senegal during drought periods: water dynamics and salt geochemistry of a developed catchment area*. Paris : ORSTOM, 263 pp. (in French).

Morillo, J.A., Antizar-Ladislao, B., Monteoliva-Sánchez, M., Ramos Cormenzana, A. & Russel, N. J. 2009. Bioremediation and biovalorisation of olive-mill wastes. *Applied Microbiology and Biotechnology* **82**, 25–39.

Moureaux Claude. 1973. *Soil microbiology course.* Bondy : ORSTOM, 163 pp. (in French).

Nasini, L., Gigliotti, G., Balduccini, M.A., Federici, E., Cenci, G. & Proietti, P. 2013. Effect of solid olive-mill waste amendment on soil fertility and olive (Olea europaea L.) tree activity. *Agric. Ecosyst. Environ*. **164**, 292–297.

Nefzaoui, A. 1985. Valuation of lignocellulosic residues in the diet of ruminants by treatment with alkali, Application to pomace oil, Catholic University of Leuven, 345 pp.

Niaounakis, M. & Halvadakis, C.P. 2006. *Olive processing waste Management: Literature Review and Patent Survey*, second edition. Elsevier Ltd., Kidlington, Oxford, UK. 514 pp.

Olsen, S.R., Cole, C.V., Watanabe, F.S. & Dean, L.A. 1954. *Estimation of available phosphorus in soils by extraction with sodium bicarbonate*. USDA Circular 939, U. S. Gov. Print, Office, Washington, DC. 19 pp.

Paredes, C, Cegarra, J, Bernal, MP & Roig, A. 2005. Influence of olive mill wastewater in composting and impact of the compost on a Swiss chard crop and soil properties. *Environ Int*. **31**, 305–12.

Proietti, P., Federici, E., Fidati, L., Scargetta, S., Massaccesi, L., Nasini, L., Regni, L., Ricci, A., Cenci, G. & Gigliotti, G. 2015. Effects of amendment with oil mill waste and its derived compost on soil chemical and microbiological characteristics and olive (Olea europaea L.) productivity. *Agric. Ecosyst. Environ*. **207**, 51–60.

Rodier Jean, Bernard Legube, Nicole Merlet. 2009. *Water Analysis*. 9th edition. Dunod, Paris. 1511 pp.

Roig, A., Cayuela, M.L. & Sánchez-Monedero, M.A. 2006. An overview on olive mill wastes and their valorisation methods. *Waste Manage* **26**, 960–969.

Secretariat of State at the Ministry of Energy, Mines, Water and Environment, in charge of Water and Environment. Quality Standards: Water for irrigation. 2007 (in French).

Walkley, A. & Black, C.A., 1934. An examination of the method for determining soil organic matter and a proposal modification of the chromic acid titration method. *Soil Science* **37**, 29–38.

Weast, R.C., Ed. 1972. *CRC Handbook of Chemistry and Physics*, 53rd Edition. Cleveland, OH:The Chemical Rubber Company Press. p. B-64.

Agronomy Research **17**(6), 2172–2179, 2019
https://doi.org/10.15159/AR.19.197

Wheat straw and lipids: UV-mutagenized *Yarrowia lipolytica* for the conversion of wheat straw hydrolysate into lipids

D. Atzmüller[*,+], F. Hawe, D. Sulzenbacher and A. Cristobal-Sarramian[+]

University of Applied Sciences Upper Austria, School of Engineering, Stelzahmerstraße 23, AT4600 Wels, Austria
[*]Correspondence: denise.atzmueller@fh-wels.at
[+]These authors contribute equally to this work

Abstract. Due to the rising energy demand of our modern society and the finite amounts of petroleum-based fuels, renewable forms of energy have become extremely important. Bio-based fuels like bioethanol and biodiesel represent an already applied and accepted alternative. Biodiesel is currently mainly produced from plant oils. A new and promising alternative, which avoids the use of food crops, is the cultivation of the oleaginous yeast *Yarrowia lipolytica*, which possesses the capacity to accumulate up to 40% of its biomass in form of lipids. Moreover, this non-conventional yeast is able to metabolize a broad range of carbon-sources, presenting a sustainable alternative to reutilize a wide spectrum of waste substrates. This makes it an auspicious candidate for the generation of non-edible oils for biodiesel production. In this work, we aimed to generate a *Y. lipolytica* mutant strain with enhanced lipid production when grown on wheat straw hydrolysate as sole carbon source. Therefore, UV mutagenesis was applied and mutants with a high-lipid content were selected by their ability to grow in the presence of the fatty acid inhibitor cerulenin. Further, growth of the mutants on wheat straw hydrolysate was evaluated. The fatty acid composition was analysed by GC-FID and the calculated total lipid content revealed an up to 33% increase compared to the wild type strain. Fermentation optimisation and the combination of various waste substrates as carbon sources are expected to further increase the total lipid yield by the *Y. lipolytica* mutant strain and serve as initial point for its industrial scale evaluation.

Key words: biodiesel, UV-mutagenesis, wheat straw hydrolysate, *Yarrowia lipolytica*.

INTRODUCTION

Fossil fuels are a finite resource unable to cover the rising energy demand of our modern society. Thus, sustainable and renewable energy sources have gained great interest during the last years and are considered key players to combat climate change and other environmental issues. After the first success of converting crops into bio-fuels, like bioethanol or biodiesel, second generation biofuels are currently being investigated due to their environmentally-friendly and sustainable character. Several waste materials are currently used as feed for biorefineries in order to produce different types of bio-based fuels. Normally, biodiesel, which is defined as the alkyl- (normally methyl- or ethyl-) esters of long chain fatty acids, is produced out of plant oil. Alternatively, production of biodiesel from the fatty acids accumulated by microorganisms like yeast represents a novel possibility in this field.

The oleaginous yeast *Yarrowia lipolytica* is a promising candidate for biodiesel production due to its capacity to accumulate up to 40% of its biomass in form of lipids (Beopoulos et al., 2009; Ageitos et al., 2011; Huang et al., 2013; Groenewald et al., 2014). Additionally, it possesses several advantages compared to plants or microalgae, which are also under investigation for this purpose (Xie, 2017). It grows much faster than microalgae and is not affected by seasonal or climate changes like plants. Furthermore, *Y. lipolytica* is able to metabolize a very broad range of different carbon-sources, which enables them to be cultivated on different waste raw materials (Ageitos et al., 2011; Groenewald et al., 2014). *Y. lipolytica* was stated as 'safe-to-use' by Groenewald et al. (2014) and is, therefore, a good candidate for economic, environmentally friendly and ethically acceptable lipid production and biodiesel generation.

Lignocellulose materials, like wheat straw, corncobs, sugar cane or rice hulls are a widely used feedstock for biorefineries worldwide (Saha, 2003; Maitan-Alfenas et al., 2015; Jönsson & Martín, 2016). Wheat straw represents the major crop residue in Europe and is only used as animal litter to a certain extent (Dias et al., 2010; Mühlenhoff, 2013). In order to utilize wheat straw as a substrate for yeast growth, two steps are required before its use: 1) Pre-treatment and 2) Hydrolysis. A detailed review about these steps is described in Cristobal-Sarramian & Atzmüller (2018).

Steam explosion is a chemical-free and very efficient type of pre-treatment to disrupt the fibre structure of wheat straw. As a result of this, the accessibility of the long chain carbon polymers, like hemicellulose, for enzymes during the hydrolysis step is improved and, thereby, the degradation of those carbon polymers into sugars is increased (mainly: glucose, galactose, xylose and mannose) (Eisenhuber et al., 2013; Marcos et al., 2013). The obtained sugar-rich solution is then further used as a carbon source during the fermentation step.

UV mutagenesis is an easy and effective method to introduce random mutations into a microorganism´s genome. The mechanisms underlying this technique have been previously described and are a suitable way to be used to generate *Y. lipolytica* mutant strains (Ikehata & Ono, 2011; Lindquist et al., 2015). For each organism and application, an optimization step is needed in order to determine the best ratio of UV-intensity and irradiation period. Additionally, the development of an adequate screening method is a critical factor for obtaining the desired strain.

For the screening of strains with enhanced fatty acid content, the fatty acid (FA) biosynthesis inhibitor cerulenin, has been shown to be an effective agent (Omura, 1976; Tapia et al., 2012; Katre et al., 2017). As cerulenin inhibits FA synthesis, it is speculated that only mutants with an enhanced FA synthesis activity will survive a lethal dose of this agent. In line with this hypothesis, Katre et al. (2017) showed, that the colony size of strains correlates with their lipid content when they are plated on cerulenin-containing media plates.

In this study, we combined the agricultural waste wheat straw as a feedstock with the capability of *Y. lipolytica* to grow on a wide range of carbon sources to produce fatty acids for biodiesel generation. Furthermore, we show, that UV mutagenesis can be used to enhance the fatty acid production and generate even more efficient strains for this purpose.

MATERIALS AND METHODS

Strains, media and growth conditions

The *Y. lipolytica* H222 strain (YlWT; *MATa* wild-type) (Barth & Gaillardin, 1996) was used as the parental strain for the UV-mutagenesis to generate the YlUV10 and YlUV12 strains. Yeast cultures were grown in YPD (yeast extract 10 g L^{-1}, peptone 20 g L^{-1}, glucose 20 g L^{-1}) or YNB medium (yeast nitrogen base without amino acids 6.7 g L^{-1}, Sigma, glucose 20 g L^{-1}). Hydrolysate (HL) medium was prepared by adding 20% wheat straw hydrolysate to YNB (6.7 g L^{-1}). Solid media was prepared by adding 2% agar to the corresponding medium. Yeast solid cultures were grown at 28 °C for up to 72 h and stored at 4 °C. For liquid cultures, precultures were prepared in 5 mL YPD and grown overnight at 28 °C on a rotary shaker (170 rpm). Prior to inoculation, precultures were washed with deionized water. Main cultures were performed in shaking flasks containing 50 mL HL-medium, inoculated with a starting OD_{600} of 0.1 and grown for 24–72 h on a rotary shaker (170 rpm). For growth characterization, this was performed three times for each strain. For lipid determination, strains were harvested after 24, 48 and 72 h by centrifugation (3,000 rpm, 10 min), washed twice with water and the cell pellets were stored at -80 °C.

UV mutagenesis and selection of strains with a high lipid content

For the UV mutagenesis, cells were grown overnight in YPD and 10 OD units were harvested, washed and resuspended in deionized water to a final volume of 10 mL. This was irradiated with UV light (254 nm) from a distance of 9 cm for 1.5 min while stirring. The killing rate was determined by harvesting cells every 30–60 s and determining their viable cell count on YPD plates in two independent experiments. Mutants with a high lipid content were selected on HL-medium plates containing 6 µg mL^{-1} cerulenin (Sigma-Aldrich, ≥ 98%). Mutants showing the biggest colonies after 48 h of incubation were further characterized.

Preparation and characterisation of wheat straw hydrolysate

Dried wheat straw was chopped to a particle size of 2–3 cm and further disintegrated by steam explosion as previously described (Eisenhuber et al., 2013). Briefly, straw was mixed with water in a 1:1 ratio and steam explosion was performed at 200 °C for 10 min. The dry weight of the steam exploded wheat straw was detected using an IR-scale (Ohaus MB45), which was immediately used for enzymatic hydrolysis. For this, wheat straw was mixed in a 1:10 (dryweight : buffer) ratio with 0.1 M citric acid buffer (pH 5.0) and 0.3 mL Accellerase 1500 (Genercor) per g dry weight. After incubation at 50 °C, 120 rpm for 72 h, big particles were removed by filtration and the liquid hydrolysate was sterile filtered and stored at 4 °C.

Saccharides, organic acids and furans from the sterile-filtered hydrolysate were quantified by HPLC, using a Jasco HPLC (2000 plus series) with an Aminex hpx 87 h column at 65 °C. H_2SO_4 (c = 5 mmol L^{-1}) as an eluent and an isocratic flow rate of 0.8 mL min^{-1} was used. Data acquisition was performed with a refractive index detection and UV-detection at 210 nm from three independently prepared samples. Data were analysed with CromPass (Version 1.8.6.1).

Fatty acid determination

Prior to analytical quantification of the fatty acid content, the washed yeast-pellets were lyophilized. Therefore a BenchTop Pro with Omnitronics (SP Scientific) freeze dryer at -50 °C (± 1) was used to dry pre-deepfreezed samples (-80 °C) for 24 h.Two times 5 mg of lyophilized yeast were resuspended and methylated separately with 5 ml of methanol : acetyl chloride (ChemLab, Sigma-Aldrich, both p.a. ≥ 99%) with a volumetric ratio of 50:2 for 8 h at 60 °C in a drying cabinet. Afterwards, the reactions were stopped by slowly adding 2.5 mL of potassium carbonate solution (VWR, ≥ 98%) with a concentration of 60 g L^{-1}. The resulting fatty acid methyl esters were extracted by adding 2 mL of hexane (Honeywell, HPLC grade) and vortexing for 1 minute. 1 mL of the supernatant phase, containing the methyl esters, was transferred in a 1.5 mL vial with a crimp cap and stored at -18 °C until measurement.

The hexane - extract was injected in a Thermo Trace 1300GC, equipped with an autosampler AS 1310 and an SSL injector. The detection was carried out by FID. The chromatographic conditions were as follows: The injection volume was 1 µL, the injector temperature was set at 240 °C. Helium was used as a carrier (constant flow) with 1.5 mL min^{-1} and a split flow at 30 mL min^{-1}. An Agilent J&W capillary column DB23 60 m, 0.25 mm ID and 0.25 µm film thickness was used for analytical separation. The oven temperature gradient was: 0–3 min 130 °C; 6.5 °C min^{-1} to 170 °C. 2.8 °C min^{-1} to 214 °C held 12 minutes. 3 °C min^{-1} to 240 °C held for 15 minutes. The FID was set at a temperature of 280 °C, 450 mL min^{-1} air flow, 45 mL min^{-1} hydrogen flow and nitrogen as make up gas at 40 mL min^{-1} were the torch conditions. Data analysis was performed with Chromeleon (Version 7.2). For calibration, the external standard method was used.

RESULTS AND DISCUSSION

Generation and selection of *Y. lipolytica* H222 mutant strains

In order to introduce mutations that lead to an improved accumulation of lipids, *Y. lipolytica* was irradiated with UV light. Prior to this mutagenesis, the optimal duration and distance for the irradiation treatment was determined. When using a lamp with a wavelength of 254 nm and a distance of 9 cm between the lamp and the cells, an exposure of 3.5 min or more lead to a kill rate greater than 90%. Sampling at 0.5, 1.5 and 2.5 min revealed kill rates of 22%, 54% and 78%, respectively (Fig. 1). Therefore, *Y. lipolytica* cultures were irradiated with UV light for 1.5 minutes to generate mutant strains.

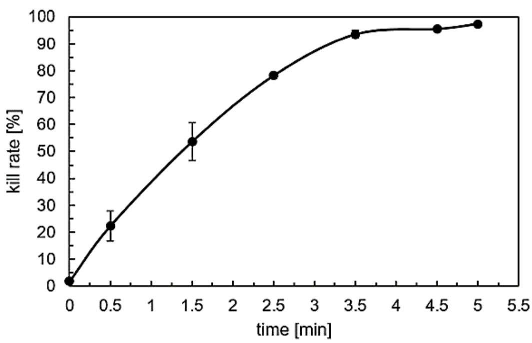


Figure 1. Determination of the kill-rate of *Y. lipolytica* H222 with UV radiation at 254 nm from a distance of 9 cm, (n = 2).

The fatty acid inhibitor cerulenin has previously been shown to be an effective selective agent to isolate yeast strains with an increased lipid content (Katre et al., 2017). Specifically, cerulenin inhibits the FA synthesis and the strains with an increased FA content tolerate a higher concentration of this agent. In order to find the appropriate cerulenin concentration for the selection of the mutants after UV treatment, growth on solid media with different cerulenin concentrations was investigated. YlWT revealed clearly inhibited growth at concentrations of 4 µg mL^{-1} and higher (Fig. 2, A). To ensure a clear selection of mutants with higher lipid content, the cells were spread onto solid HL-media containing 6 µg mL^{-1} cerulenin. The colonies with the biggest diameter were picked from the plate after 48 h incubation and transferred to a new cerulenin selection plate. After a second selection step on the same media, the mutants YlUV10 and YlUV12 were chosen as potential candidates with an increased lipid content, based on their growth and colony morphology (Fig. 2, B). Usually, UV-mutagenesis experiments are associated with the development of so-called *petite* phenotypes, which are linked to mitochondrial dysfunction and delayed growth (Chanet & Heude, 1974). Thus, in order to avoid the selection of strains with possible growth defects, which would result in a decrease fermentation productivity, we assumed that the strains with the biggest colony sizes would not have a growth defect and were further characterized in this study.

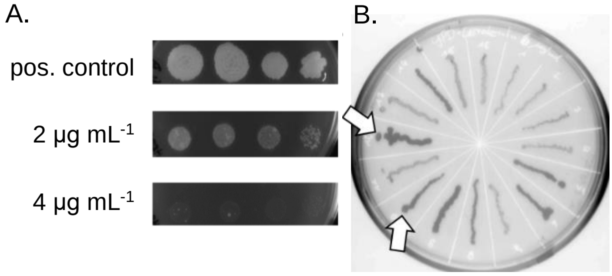


Figure 2. A: Evaluation of the toxic concentration of cerulenin for YlWT after 48 h, pos. control = YPD; B: Second selection step of *Y. lipolytica* mutants after UV mutagenesis on HL-media plates containing 6 µg mL^{-1} cerulenin, selected mutants (YlUV10 and YlUV12) are highlighted with arrows.

Characterisation of wheat straw hydrolysate and growth evaluation

In order to use wheat straw as carbon source for *Y. lipolytica* cultivation, a pre-treatment step of this raw material is required to obtain a fermentable sugar solution. This was achieved by steam-explosion and enzymatic hydrolysis. Several steam-explosion parameters were tested in order to achieve the highest sugar content after enzymatic hydrolysis (unpublished data). HPLC analysis of the wheat straw hydrolysate revealed that steam explosion performed at 200 °C for 10 minutes resulted in the highest sugar concentration (Table 1). Although there are growth inhibitors released during this process, their concentrations were not sufficient to impair *Y. lipolytica* growth (unpublished data), which is also comparable with the findings of Niehus et al. (2018).

Table 1. Sugar and inhibitor concentrations of wheat straw hydrolysate obtained after steam explosion (200 °C, 10 mins) and enzymatic hydrolysis, (n = 3)

Sugars	Concentration, g L^{-1}	Inhibitors	Concentration, g L^{-1}
Cellobiose	1.82 (± 0.27)	Formic acid	4.28 (± 0.16)
Glucose	32.48 (± 0.59)	Acetic acid	4.09 (± 0.21)
Xylose/ Mannose	13.02 (± 1.25)	Furfural	0.27 (± 0.27)

Growth of the mutants was evaluated in HL-medium and compared with the YlWT strain to ensure that the UV treatment did not affect the strain's ability to grow on wheat straw hydrolysate as the sole carbon source. The growth evaluation shows a similar growth behaviour in all three strains (Fig. 3). This indicates that wheat straw is a suitable raw material to use as a media to grow *Y. lipolytica*, and the introduced mutations did not compromise any essential pathway affecting yeast growth. Therefore, the mutants were further characterized for FA production in HL-medium.

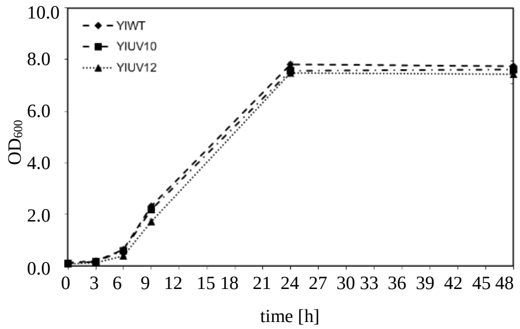


Figure 3. Growth evaluation of YlWT (◆) and the two mutants YlUV10 (■) and YlUV12 (▲) in HL-media, (n = 3).

Fatty acid production

For the comparison of the FA production of the generated mutant strains, YlUV10 and YlUV12, with YlWT, all strains were grown in HL-media and harvested after 24 h, 48 h and 72 h. Samples were freeze dried and fatty acid methylation was performed prior to GC analysis. Interestingly, the total FA content of all three analysed strains was clearly different (Fig. 4). On the one hand, YLUV10, in comparison with YlWT, showed lower values after 24 h and 72 h (-16% and -8%, respectively), but an about 33% higher content after 48 h. On the other hand, YlUV12 showed the opposite behaviour, here after 24 h and 72 h the total FA content is slightly higher (9% each) than for YlWT, but lower (-9%) after 48 h.

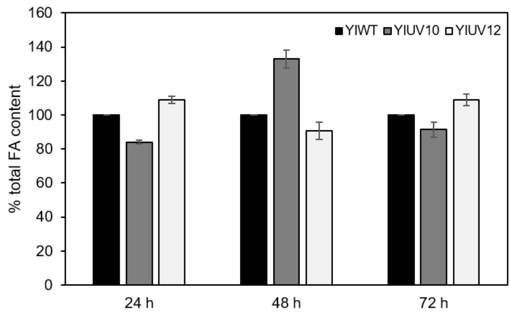


Figure 4. Fatty acid content of YlUV10 and YlUV12 after 24 h, 48 h and 72 h cultivation in shaking flasks with HL-media compared to the YlWT (set to 100%), (n = 2).

The fact that the YlUV10 strain showed an increased FA-content after 48 h, which then decreases after 72 h, indicates that the activity of the FA synthesis enzymes is higher during the first 48 h, which correlates with the enhanced cerulenin resistance of this strain. In case of the YlUV12, the FA content is slightly increased after 72 h in comparison to YlWT. Interestingly, this strain did not show any growth defect in comparison to YlWT. Thus, one may speculate that the differences within this time frame are caused by intensive lipid remodelling events, which are a major event influencing overall yeast physiology (Henry et al., 2012; Renne et al., 2015; Sarria et al., 2017). Another possibility could be associated to an altered sugar uptake or utilization in the mutants, which even if a direct impact on cellular growth was not observed, could have an influence on the total lipid content of these strains (Hapeta et al., 2017; Tanimura et al., 2018). Further investigation exploring these hypotheses, as well as other possible molecular mechanism behind the lipid accumulation in these mutants strains will be conducted.

CONCLUSIONS

In the present study, we generated *Y. lipolytica* strains with an enhanced lipid content when grown on wheat straw hydrolysate. This was achieved by combining UV-mutagenesis, cerulenin screening and adaptation to wheat straw hydrolysate experiments. Nowadays, metabolic engineering is a widely accepted technology to increase *Y. lipolytica* lipid content (Bhutada et al., 2017). Thus, it will be interesting to apply this approach to further increase the lipid content of the Yl10UV and Yl12UV strains, for instance by modulation the activity of the FA synthesis and neutral lipid hydrolysing enzymes. Furthermore, the ability of *Y. lipolytica* to metabolize different carbon sources provides a great opportunity to use this yeast in biorefineries (Kavšček et al., 2015). Further growth characterization of this yeast on substrates commonly used in biorefineries as well as the optimization of the fermentation parameters will serve as a starting point to evaluate the use of these strains in an industrial scale level.

ACKNOWLEDGEMENTS. This work (project 'Combined Agro-Forest Biorefinery – CAFB') was supported within the scope of the European Union Program 'IWB/EFRE-Program 2014–2020' from the purse of the European Regional Development Fund (ERDF) and the Federal State of Upper Austria.

REFERENCES

Ageitos, J.M., Vallejo, J.A., Veiga-Crespo, P. & Villa, T.G. 2011. Oily yeasts as oleaginous cell factories. *Appl. Microbiol. Biotechnol.* **90**(4), 1219–1227.

Barth, G. & Gaillardin, C. 1996. Yarrowia lipolytica. In *Nonconv. Yeasts* (pp. 313–88). Springer Berlin Heidelberg.

Beopoulos, A., Cescut, J., Haddouche, R., Uribelarrea, J.-L., Molina-Jouve, C. & Nicaud, J.-M. 2009. Yarrowia lipolytica as a model for bio-oil production. *Prog. Lipid Res.* **48**(6), 375–387.

Bhutada, G., Kavšček, M., Ledesma-Amaro, R., Thomas, S., Rechberger, G.N., Nicaud, J.-M. & Natter, K. 2017. Sugar versus fat: Elimination of glycogen storage improves lipid accumulation in Yarrowia lipolytica. *FEMS Yeast Res.* **17**(3), 1–10.

Chanet, R. & Heude, M. 1974. Variations in UV induced lethality and "petite" mutagenesis in synchronous culture of Saccharomyces cerevisiae. *Mol. Gen. Genet.* **131**(1), 21–26.

Cristobal-Sarramian, A. & Atzmüller, D. 2018. Yeast as a production platform in biorefineries: Conversion of agricultural residues into value-added products. *Agron. Res.* **16**(2), 377–388.

Dias, A.A., Freitas, G.S., Marques, G.S.M., Sampaio, A., Fraga, I.S., Rodrigues, M.A.M., … Bezerra, R.M.F. 2010. Enzymatic saccharification of biologically pre-treated wheat straw with white-rot fungi. *Bioresour. Technol.* **101**(15), 6045–6050.

Eisenhuber, K., Krennhuber, K., Steinmüller, V. & Jäger, A. 2013. Comparison of different pre-treatment methods for separating hemicellulose from straw during lignocellulose bioethanol production. *Energy Procedia* **40**, 172–181.

Groenewald, M., Boekhout, T., Neuvéglise, C., Gaillardin, C., Van Dijck, P.W.M. & Wyss, M. 2014. Yarrowia lipolytica: Safety assessment of an oleaginous yeast with a great industrial potential. *Crit. Rev. Microbiol*. **40**(3), 187–206.

Hapeta, P., Rakicka, M., Dulermo, R., Gamboa-Meléndez, H., Cruz-Le Coq, A.-M., Nicaud, J.-M. & Lazar, Z. 2017. Transforming sugars into fat - lipid biosynthesis using different sugars in Yarrowia lipolytica. *Yeast* **34**(April), 1–25.
Henry, S.A., Kohlwein, S.D. & Carman, G.M. 2012. Metabolism and regulation of glycerolipids in the yeast Saccharomyces cerevisiae. *Genetics* **190**(2), 317–349.
Huang, C., Chen, X., Xiong, L., Chen, X., Ma, L. & Chen, Y. 2013. Single cell oil production from low-cost substrates: The possibility and potential of its industrialization. *Biotechnol. Adv.* **31**(2), 129–139.
Ikehata, H. & Ono, T. 2011. The Mechanisms of UV Mutagenesis. *J. Radiat. Res.* **52**(2), 115–125.
Jönsson, L.J. & Martín, C. 2016. Pretreatment of lignocellulose: Formation of inhibitory by-products and strategies for minimizing their effects. *Bioresour. Technol.* **199**, 103–112.
Katre, G., Ajmera, N., Zinjarde, S. & RaviKumar, A. 2017. Mutants of Yarrowia lipolytica NCIM 3589 grown on waste cooking oil as a biofactory for biodiesel production. *Microb. Cell Fact.* **16**(1), 176.
Kavšček, M., Stražar, M., Curk, T., Natter, K. & Petrovič, U. 2015. Yeast as a cell factory: Current state and perspectives. *Microb. Cell Fact.* **14**(1), 1–10.
Lindquist, M.R., López-Núñez, J.C., Jones, M.A., Cox, E.J., Pinkelman, R.J., Bang, S.S., … Hughes, S.R. 2015. Irradiation of Yarrowia lipolytica NRRL YB-567 creating novel strains with enhanced ammonia and oil production on protein and carbohydrate substrates. *Appl. Microbiol. Biotechnol.* **99**(22), 9723–9743.
Maitan-Alfenas, G.P., Visser, E.M. & Guimarães, V.M. 2015. Enzymatic hydrolysis of lignocellulosic biomass: Converting food waste in valuable products. *Curr. Opin. Food Sci.* **1**(1), 44–49.
Marcos, M., García-Cubero, M.T., González-Benito, G., Coca, M., Bolado, S. & Lucas, S. 2013. Optimization of the enzymatic hydrolysis conditions of steam-exploded wheat straw for maximum glucose and xylose recovery. *J. Chem. Technol. Biotechnol.* **88**(2), 237–246.
Mühlenhoff, J. 2013. Reststoffe für Bioenergie nutzen - Potenziale, Mobilisierung und Umweltbilanz. *Renews Spez.* **64**(64), 1–46.
Niehus, X., Crutz-Le Coq, A.M., Sandoval, G., Nicaud, J.M. & Ledesma-Amaro, R. 2018. Engineering Yarrowia lipolytica to enhance lipid production from lignocellulosic materials. *Biotechnol. Biofuels* **11**(1), 1–10.
Omura, S. 1976. The antibiotic cerulenin, a novel tool for biochemistry as an inhibitor of fatty acid synthesis. *Bacteriol. Rev.* **40**(3), 681–697.
Renne, M.F., Bao, X., De Smet, C.H. & de Kroon, A.I.P.M. 2015. Lipid acyl chain remodeling in yeast. *Lipid Insights* **8**, 33–40.
Saha, B.C. 2003. Hemicellulose bioconversion. *J. Ind. Microbiol. Biotechnol.* **30**(5), 279–291.
Sarria, S., Kruyer, N.S. & Peralta-Yahya, P. 2017. Microbial synthesis of medium-chain chemicals from renewables. *Nat. Biotechnol.* **35**, 1158.
Tanimura, A., Sugita, T., Endoh, R., Ohkuma, M., Kishino, S., Ogawa, J., … Takashima, M. 2018. Lipid production via simultaneous conversion of glucose and xylose by a novel yeast, Cystobasidium iriomotense. *PLoS One* **13**(9), 1–13.
Tapia, E.V, Anschau, A., Coradini, A.L., T Franco, T. & Deckmann, A. 2012. Optimization of lipid production by the oleaginous yeast Lipomyces starkeyi by random mutagenesis coupled to cerulenin screening. *AMB Express* **2**(1), 64.
Xie, D. 2017. Integrating Cellular and Bioprocess Engineering in the Non-Conventional Yeast Yarrowia lipolytica for Biodiesel Production: A Review. *Front. Bioeng. Biotechnol.* **5**(October).

Agronomy Research **17**(6), 2180–2186, 2019
https://doi.org/10.15159/AR.19.165

The potential of energy recovery from by–products of small agricultural farms in Nigeria

M. Bappah*, J. Bradna, J. Velebil and J. Malatak

Czech University of Life Sciences, Faculty of Engineering, Department of Technological Equipment of Buildings, Kamycka 129, CZ16521 Praha 6–Suchdol, Prague, Czech Republic
*Correspondence: bappahmusa@gmail.com

Abstract. Agricultural by–products are renewable energy sources from which essential amount of energy can be recovered, which can be used to replace the use of conventional fossil fuel, reduces the potential of greenhouse gas (GHG) emission and at the same time reduces deforestation, especially in rural areas. Energy values of biomass from small Agricultural farms, in particular waste generated from different tropical crops, viz; Maize, Millet, Rice, Sorghum and Groundnut were determined, to ascertain their potentials as alternative fuel sources for rural use. The materials were found to be of importance judging by their combustion potentials in all the forms investigated. The Energy values of the by-products considered ranged between 11.68 MJ kg^{-1} to 17.48 MJ kg^{-1} with Groundnut pods and millet husk having the highest and least respectively. Moisture and ash had effect on the energy values of these biomass. Our results are relevant to the problems posed by the management of farm residues in developing countries.

Key words: by–products, energy, tropical biomass.

INTRODUCTION

Global increase in human population leads to an increase in Agricultural production, which as well results to rise in the production of Agricultural by–products at both farm and industrial levels.

Agricultural waste comprises both natural (organic) and non–natural wastes which can either be solid, liquid and slurries resulting from growing and first processing of Agricultural products. Uncontrolled or improper handling of which may lead to environmental pollution (Zhang et al., 2012). Crop residues include: straw from barley, rice, soy bean and wheat; Stover from maize; bagasse from sugar cane (Bentsen et al., 2014), rice husk, corn cobs, cocoa pods, fruits shell (Titiloye et al., 2013), sorghum and millet husk and groundnut pods.

Potential energy can be derived from Agricultural wastes which are produced through Agricultural practices (Bentsen et al., 2014). The type of waste, its quantity and geographical location, and also the handling practice by local farmers determines the ways through which the energy can be recovered (Jana & De, 2015). Energy values of different Agricultural wastes vary and this informs the methods and techniques adopted in their use (Titiloye et al., 2013). Physico–chemical characteristics of such materials offer means for such assessment (McKendry, 2002b). For example, rice straw is used as cattle fodder and may be burnt or gasified for heating or electricity generation (Jana & De, 2015). Renewable sources of energy offer a means for mitigating and reducing the effects of global warming (McKendry, 2002a).

Maize, rice, millet, Sorghum and groundnut are among the highly produced crops in Nigeria from which different by–products can be obtained which are of importance as biomass feedstock. Relative yield of maize cobs is between 10% and 20% of total crop mass, at 10%–12% moisture levels, normally left on farms after threshing. In rural areas of developing countries, such perceived by–product may be utilized for direct combustion purposes (Martinov et al., 2011).

Maize is the most widely cultivated cereal crop with estimated global annual production of 717 metric tons (Ranum et al., 2014). Maize cobs which comprise 18.7% of the total grain mass (Blandino et al., 2016), are currently being used for heating in some parts of Europe (F. John Hay, 2015) and as animal bedding. Rice with an estimated global annual production of 120 million tons (Abbas & Ansumali, 2010), whose husk is essentially 20% of the crop's volume is seldom considered to be fodder since it is rich in silica and decomposes slowly. Through direct combustion, this by–product may be utilized for heating and power generation (Dunnigan et al., 2018). Millet is a subsistence crop mostly cultivated for local consumption, with an estimated global production of 28 tons year^{-1} (FAO and ICRISAT, 1996). Millet husks left on the farm after harvest often become hosts for crop disease pathogens and it may be helpful to modify them for combustion (Abba et al., 2017). Groundnut (*Arachis hypogaea* L.) is one of the most important cash crops of West Africa and a source of edible vegetable oil, adaptable also for biofuel purposes (Baributsa et al., 2017). Its global annual production was estimated as 34 million metric tons. Nautiyal (2002) and the pods are 20%–30% by weight of the seed crop, often discarded or burnt after threshing, constituting serious pollution problem (Deeba et al., 2017). With nearly 57 million tons of annual production, sorghum is a rich source of easily sourced, inexpensive and readily available biomass feedstock (Monteiro et al., 2012; Wizi et al., 2018).

Over 2.5 billion people depends on traditional use of biomass as their primary fuel for cooking in developing countries (Toklu, 2017), transformation of which will be more efficient and bio friendly. Biomass with low moisture content are more appropriate for thermal conversion technology, whereas those with high moisture content are recommended for fermentation and anaerobic digestion (Garivait et al., 2006).

The by-products are often discarded or burn in the farm thereby polluting the environment and contributing to global warming, knowing the energy value of which will help in determining appropriate way of management for value addition.

MATERIALS AND METHODS

Materials

Tropical agricultural by–products which were dried husks of rice, millet and sorghum, pods of groundnut and cobs of maize at moisture content of less than 10%, in dry basis obtained from different parts of Bauchi state, Nigeria were used. A representative sample of each was collected and transported to Czech Republic for the study. Three replications of each test were carried out and the mean values were reported. The energy potential of which were determined under two different states; as received and dry basis.

Methods

The moisture content of the raw by–products were determined according to EN ISO 18134–3:2015, using Memmert UF30 laboratory oven at 105ºC, which were calculated using equation (1) (Havrland et al., 2013; Pňakovič & Dzurenda, 2015).

$$w = \left(\frac{m_0 - m_1}{m_0}\right) \cdot 100 \quad (1)$$

where w – moisture content; m_0 – mass of the samples before drying and m_1 – mass of the samples after drying.

Samples were milled to 1mm screen fraction, using Retsch SM100 Milling Machine. An automated oven, LECO TGA701 was used in determining moisture and ash contents of the biomass, in accordance with the EN ISO 18122:2015 standard (Ivanova et al., 2018). Higher Heating Value of the biomass were measured according to ISO 1928: 2009 standard, using LECO AC 600 Calorimeter. Lower Heating Value of dry basis of the biomass samples were calculated using Eq. (2) (Pňakovič & Dzurenda, 2015).

$$LHV = \left(HHV - 212w_{Hd} - 0.8 \cdot (w_{Od} + w_{Hd})\right) \cdot (1 - 0.01M_t) - 24.43M_T \quad (2)$$

where LHV – lower heating value (MJ kg^{-1}); HHV – higher heating value (MJ kg^{-1}); w_{Od} – oxygen content in dry state (%wt.); w_{Hd} – hydrogen content in dry state (%wt.); M_T – target moisture (0% for dry state).

Ultimate analysis of the biomass were then carried out to determine C, H, O, N and S content (McKendry, 2002a; Ivanova et al., 2018) using Leco CHN628/628 S.

RESULTS AND DISCUSSION

Proximate and ultimate compositions of as received and dry samples of different biomass materials studied are presented in Tables 1 and 2 along with their associated measures of uncertainty at confidence levels of 95%.

The moisture level of the by–products sampled was approximately 7% in dry basis. For the samples analyzed in as received basis, Groundnut pods and Rice husk had the highest and lowest heating values (Table 1). Ash content in rice husks and millet husk was 22% and 30% respectively, which are higher than the levels observed in other samples whose ash contents were below 10% (Table 1). Higher ash content can be attributed to contamination with sand or dust particles during threshing and sample collection (Titiloye et al., 2013; Pňakovič & Dzurenda, 2015).

Carbon content was higher in the samples with low ash content. Energy values were also higher in materials with low ash content and high carbon content (Table 1). Energy values of the by–products considered ranged between 11.68–17.48 MJ kg^{-1}. Groundnut pods had significantly higher energy values, compared to other materials (Table 1).

With the elimination of moisture from product samples, Carbon contents improved significantly, though, no significant change in ash contents was observed for all of the biomass tested (Table 2). Lower moisture content of the biomass favor better thermal conversion (Demirbas, 2007; Titiloye et al., 2013; Palackaa et al., 2017).

Table 1. Proximate and ultimate composition of the analysed biomass together with their respective uncertainties

Sample	w %wt.	A %wt.	C %wt.	H incl. water %wt.	H in combustable %wt.	N %wt.	S %wt.	O %wt.	HHV MJ kg^{-1}	LHV MJ kg^{-1}
Rice	6.63 ± 0.11	22.02 ± 0.15	35.96 ± 0.38	5.37 ± 0.06	4.61 ± 0.06	0.86 ± 0.03	< 0.05	29.91 ± 0.42	14.48 ± 0.04	13.32 ± 0.05
Sorghum	7.26 ± 0.09	8.42 ± 0.69	42.29 ± 0.57	5.78 ± 0.08	4.97 ± 0.08	0.41 ± 0.07	< 0.05	36.65 ± 0.90	15.93 ± 0.28	14.66 ± 0.16
Groundnut	7.92 ± 0.07	3.19 ± 0.25	47.68 ± 0.26	6.14 ± 0.07	5.26 ± 0.07	1.14 ± 0.05	< 0.05	34.83 ± 0.37	18.81 ± 0.07	17.48 ± 0.07
Maize	7.56 ± 0.05	1.66 ± 0.17	45.54 ± 0.26	6.16 ± 0.07	5.32 ± 0.07	0.41 ± 0.05	< 0.05	39.52 ± 0.33	17.59 ± 0.16	16.25 ± 0.16
Millet	5.37 ± 0.24	30.43 ± 2.35	33.34 ± 1.48	4.45 ± 0.21	3.85 ± 0.18	0.85 ± 0.12	< 0.05	26.36 ± 1.94	12.65 ± 0.48	11.68 ± 0.48

w – moisture content; A – ash content; C – carbon content; H – hydrogen content; N – nitrogen content; S – sulfur content; O – oxygen content; HHV – higher heating value; LHV – lower heating value.

Table 2. Composition of dry biomass

Sample	A^d %wt.	C^d %wt.	H^d %wt.	N^d %wt.	S^d %wt.	O^d %wt.	HHV MJ kg^{-1}	LHV MJ kg^{-1}
Rice	23.58 ± 0.16	38.52 ± 0.41	4.94 ± 0.07	0.92 ± 0.03	< 0.05	32.04 ± 0.45	15.52 ± 0.05	14.44 ± 0.05
Sorghum	9.08 ± 0.74	45.60 ± 0.62	5.36 ± 0.08	0.44 ± 0.08	< 0.05	39.52 ± 0.97	17.17 ± 0.17	16.00 ± 0.17
Groundnut	3.46 ± 0.27	51.78 ± 0.28	5.71 ± 0.07	1.24 ± 0.05	< 0.05	37.82 ± 0.40	20.44 ± 0.08	19.19 ± 0.08
Maize	1.79 ± 0.19	49.26 ± 0.29	5.75 ± 0.07	0.44 ± 0.05	< 0.05	42.75 ± 0.35	19.03 ± 0.17	17.78 ± 0.17
Millet	32.16 ± 2.48	35.23 ± 1.56	4.07 ± 0.20	0.89 ± 0.13	< 0.05	27.85 ± 2.04	13.37 ± 0.50	12.48 ± 0.51

A^d – ash content in dry state; C^d – carbon content in dry state; H^d – hydrogen content in dry state; N^d – nitrogen content in dry state; S^d – sulfur content in dry state; O^d – oxygen content in dry state; HHV – higher heating value; LHV – lower heating value.

Inorganic residue ash of the dry biomass with their respective heating values and ultimate composition are presented in Table 2. Ash content of maize cobs was the least (1.79%wt). Ash content of rice husks was 23.58%; this is less than the value reported by (Titiloye et al., 2013) but comparable to that of commercial coal which falls within the range of 5%–20%wt (Palackaa et al., 2017). In this state, energy values of the pods of groundnut were the highest, being 19.19 MJ kg^{-1} while those of dried millet husks stood at 12.48 MJ kg^{-1}, comparable to values reported in similar works (Titiloye et al., 2013). These values represent significant proportions and potentials for thermal conversion.

Nitrogen levels in the feedstock were low, which indicates a favorable risk of associated oxide emission during combustion (Pňakovič & Dzurenda, 2015). Sulfur contents were also negligible.

The energy value of all the by–products tested increases with decreasing moisture content (Demirbas, 2007; Titiloye et al., 2013; Palackaa et al., 2017; Paudel et al., 2017), which can be attributed to low carbon and oxygen content of the undried samples.

The energy value of Groundnut shell and Rice husk are higher than what was reported in similar researches as presented in Table 3. Similar values were discovered by Demirbas (2007) for heating value of Maize cobs, with other reported values less than what was obtained in this research (Table 3).

Table 3. Heating value of Agricultural by-products compared with previous researches

Sample	w %wt.	A %wt.	HHV MJ kg^{-1}	LHV MJ kg^{-1}	Source
Rice husk	**6.63**	**22.02**	**14.48**	**13.32**	
	8.59	24.71	14.08	-	(Titiloye et al., 2013)
Sorghum husk	**7.26**	**8.42**	**15.93**	**14.66**	
Groundnut pods	**7.92**	**3.19**	**18.81**	**17.48**	
	12	3.47	17.12	-	(Oyelaran, 2014)
	10.3	6.0	16.4	-	(Onuegbu et al., 2012)
Maize cobs	**7.56**	**1.66**	**17.59**	**16.25**	
	8.72	2.96	16.24	-	(Titiloye et al., 2013)
	5.7	1.3	17.7	16.2	(Demirbas, 2007)
	12.2	3.3	16.4	-	(Onuegbu et al., 2012)
Millet husk	**5.37**	**30.43**	**13.37**	**12.48**	

w – moisture content; A – ash content; HHV – higher heating value; LHV – lower heating value.

The by-products can either be combusted directly or transformed into different forms of biofuel (solid liquid or gas) using different conversion technologies (Toklu, 2017). Improvement through pelletization can reduce moisture content, increase bulk density and as well increases the heating value (Rosillo-Calle et al., 2007; Jagustyn et al., 2011), which will ensure efficient utilization in rural areas.

CONCLUSIONS

The energy value of some tropical Agricultural by–products were investigated under two different states. Heating values increases with decreasing moisture content and decreases with increasing ash content. Carbon and oxygen also increases with decreasing moisture content. Dry basis of all the samples tested has the highest heating value, while as received basis has the least. The biomass have favorable heating values under all the two states, they can therefore be efficiently used for combustion through pelletization.

ACKNOWLEDGEMENTS. This study was supported by the Internal Grant Agency of the Faculty of Engineering, Czech University of Life Science Prague, grant number: 2018:31170/1312/3123.

REFERENCES

Abba, H.A., Zahari, I.N., Sapuan, S.M. & Leman, Z. 2017. Characterization of Millet (Pennisetum glaucum) Husk Fiber (MHF) and Its Use as Filler for High Density Polyethylene (HDPE) Composites. *Bioresources* **12**, 9287–9301.

Abbas, A. & Ansumali, S. 2010. Global Potential of Rice Husk as a Renewable Feedstock for Ethanol Biofuel Global Potential of Rice Husk as a Renewable Feedstock for Ethanol Biofuel Production. *BioEnergy Research* **3**, 328–334. https://doi.org/10.1007/s12155-010-9088-0

Baributsa, D., Baoua, I.B., Bakoye, O.N., Amadou, L. & Murdock, L.L. 2017. PICS bags safely store unshelled and shelled groundnuts in Niger. *Journal of Stored Products Research* **72**, 54–58. https://doi.org/10.1016/j.jspr.2017.03.007

Bentsen, N.S., Felby, C. & Thorsen, B.J. 2014. Agricultural residue production and potentials for energy and materials services. *Progress in Energy and Combustion Science* **40**, 59–73. https://doi.org/10.1016/J.PECS.2013.09.003

Blandino, M., Fabbri, C., Soldano, M., Ferrero, C., Reyneri, A., Sciences, F. & To, G. 2016. The use of cobs, a maize grain by-product, for energy production in anaerobic digestion. *Italian Journal of Agronomy* **11**(754), 195–198. https://doi.org/10.4081/ija.2016.754

Deeba, F., Pruthi, V. & Negi, Y.S. 2017. Fostering triacylglycerol accumulation in novel oleaginous yeast Cryptococcus psychrotolerans IITRFD utilizing groundnut shell for improved biodiesel production. *Bioresource Technology* **242**, 113–120. https://doi.org/10.1016/j.biortech.2017.04.001

Demirbas, A. 2007. Environmental Effects of Moisture and Hydrogen Content on the Heating Value of Fuels. *Energy sources* **29**, 649–655. https://doi.org/10.1080/009083190957801

Dunnigan, L., Ashman, P.J., Zhang, X. & Kwong, C.W. 2018. Production of biochar from rice husk: Particulate emissions from the combustion of raw pyrolysis volatiles. *Journal of Cleaner Production* **172**, 1639–1645. https://doi.org/10.1016/j.jclepro.2016.11.107

EN ISO 18122:2015. (2015). *Solid biofuels – Determination of ash content*. Bonn, Germany: BSI Standards Publication.

EN ISO 18134-3:2015. (2015). *Solid biofuels – Determination of moisture content – Oven dry method* (Part 3: Mo). Bonn, Germany: BSI Standards Publication.

F. John Hay. 2015. Corn for Biofuel Production Current Potential for Use as a Biofuel. *Farm Energy*, 3–5.

FAO and ICRISAT. 1996. *The world sorghum and millet economies*, 31 pp.

Garivait, S., Chaiyo, U. & Patumsawad, S. 2006. Physical and chemical properties of Thai biomass fuels from agricultural residues. In *2nd Joint International Conference on "Sustainable Energy and Environment (SEE 2006)."* Bangkok, Thailand, pp. 41–48.

Havrland, B., Ivanova, T. & Kolarikova, M. 2013. Comparative analysis of bio-raw materials and biofuels. *Engineering for rural development*, pp. 541–544.

ISO 1928. 2009. Solid Mineral fuels-Determination of Gross calorific value by the bomb calorimetric method and calculation of Net calorific value, *2009*.

Ivanova, T., Muntean, A., Havrland, B. & Hutla, P. 2018. Quality assessment of solid biofuel made of sweet sorghum biomass Quality assessment of solid biofuel made of sweet sorghum biomass. *Contemporary Research Trends in Agricultural Engineering* **10**, 1–5. https://doi.org/10.1051/bioconf/20181002007

Jagustyn, B., Bątorek-giesa, N., Wilk, B. & Processing, C. 2011. Evaluation of properties of biomass used for energy purposes. *Science technique* **65**(6), 560–563.

Jana, K. & De, S. 2015. Polygeneration using agricultural waste: Thermodynamic and economic feasibility study. *Renewable Energy* **74**, 648–660. https://doi.org/10.1016/j.renene.2014.08.078

Martinov, M.L., Veselinov, B.V., Bojić, S.J. & Djatkov, D.M. 2011. Investigation of maize cobs crushing - Preparation for use as a fuel. *Thermal Science* **15**(1), 235–243. https://doi.org/10.2298/TSCI100407059M

McKendry, P. 2002a. Energy production from biomass (part 1): overview of biomass. *Bioresource Technol* **83**(1), 37–46. https://doi.org/10.1016/S0960-8524(01)00118-3

McKendry, P. 2002b. Energy production from biomass (part 2): Conversion technologies. *Bioresource Technology* **83**(1), 47–54. https://doi.org/10.1016/S0960-8524(01)00119-5

Monteiro, J., Havrland, B., Ivanova, T. & Agriculture, S. 2012. An Integrated View of Sweet Sorghum (Sorghum bicolor (L.) Moench) Bioenergy Value – Importance for Portugal. *Agricultura tropica et subtropica* **45**(1), 12–19. https://doi.org/10.2478/v10295-012-0002-y

Nautiyal, P.C. 2002. *GROUNDNUT Post-harvest Operations*. (AGSI/FAO, Ed.), *Food and Agricultural Organization of the United Natiion*, pp. 5

Onuegbu, T.U., Ilochi, N.O., Ogbu, I.M., Obumselu, F.O. & Okafor, I. 2012. Preparation of Environmental Friendly Bio-coal Briquette from Groundnut Shell and Maize Cob Biomass Waste: Comparative Effects of Ignition Time and Water Boiling Studies. *Current Research in Chemistry* **4**, 110–118. https://doi.org/10.3923/crc.2012.110.118

Oyelaran, O. 2014. Evaluating the bio-energy potential of groundnut shell and sugarcane bagasse waste composite. *Journal of Renewable Energy and Environment* **42**, 306–310. https://doi.org/10.14456/kkuenj.2015.36

Palackaa, M., Viciana, P., Holubčíka, M. & Jandačkaa, J. 2017. The energy characteristics of different parts of the tree. *12th International Scientific Conference on Sustainable, Modern and Safe Transport* **192**, pp. 654–658. Retrieved from https://www.sciencedirect.com/science/article/pii/S1877705817326590

Paudel, S.R., Banjara, S.P., Choi, O.K., Park, K.Y., Kim, Y.M. & Lee, J.W. 2017. Pretreatment of agricultural biomass for anaerobic digestion: Current state and challenges. *Bioresource Technology* **245**, 1194–1205. https://doi.org/10.1016/j.biortech.2017.08.182

Pňakovič, Ľ. & Dzurenda, L. 2015. Combustion Characteristics of Fallen Fall Leaves from Ornamental Trees in City and Forest Parks. *BioResources* **10**(3), 5563–5572.

Ranum, P., Pena-Rosas, J. & Garcia-Casal, M.N. 2014. Global maize production, utilization, and consumption. *Annals of the New York Academy of Sciences* **1312**, pp. 5563–5572. https://doi.org/10.1111/nyas.12396

Rosillo-Calle, F., Groot, P. de, Hemstock, S.L. & Woods, J. (Eds.). 2007. *The Biomass Assessment Handbook* (First). London: Earthscan publishers, pp. 126–126.

Titiloye, J.O., Abu Bakar, M.S. & Odetoye, T.E. 2013. Thermochemical characterisation of agricultural wastes from West Africa. *Industrial Crops and Products* **47**, 199–203. https://doi.org/10.1016/j.indcrop.2013.03.011

Toklu, E. 2017. Biomass energy potential and utilization in Turkey. *Renewable Energy* **107**, 235–244. https://doi.org/10.1016/j.renene.2017.02.008

Wizi, J., Wang, L., Hou, X., Tao, Y., Ma, B. & Yang, Y. 2018. Industrial Crops & Products Ultrasound-microwave assisted extraction of natural colorants from sorghum husk with di ff erent solvents. *Industrial Crops & Products* **120**, 203–213. https://doi.org/10.1016/j.indcrop.2018.04.068

Zhang, Z., Gonzalez, A.M., Davies, E.G.R. & Liu, Y. 2012. Agricultural Wastes. *Water Environment Research* **84**(10), 1386–1406. https://doi.org/10.2175/106143012X13407275695193

Agronomy Research **17**(6), 2187–2194, 2019
https://doi.org/10.15159/AR.19.219

Germination of proso millet (*Panicum miliaceum L.*) grains trigger biochemical changes that augment bioavailability of flower and its utility for gluten-free dietary foods

I.A. Bazhenova, T.S. Bazhenova* and E.V. Chernova

Peter the Great Polytechnic University, The Graduate School of Biotechnology and Food Technology, street Novorossiyskaya, 48, RU194021 St-Petersburg, Russia
*Correspondence: tatjanabazhenova@mail.ru

Abstract. During past decade, there has been an active search for new sources and means, such as biologic modification of raw plant material, to produce bioavailable foods with pre-defined properties. In this study, we tested whether germination of proso millet (*Panicum miliaceum L.*) grains could be used to increase bioavailability of the flower for gluten-free diets (GFD). Our analysis demonstrated that grains of four selected cultivars had similar germination rates in different media. However, the pikes of amylolytic and proteinase activities were detected at the 2nd and 3rd days of germination, whereas the pike of lipase activity was associated with the 4th day. The highest and the lowest enzymatic activities were detected in grains germinated in whey and in NaCl, respectively. During germination, cumulative phenolic content increased up to 3.5 times reaching the highest levels by day 5. Based on these data, we produce batches of flour from grains germinated for 3 days and evaluated its utility in producing non-rising cake and shortbread pastry dough. Sensory evaluation of the baked products confirmed that flour from germinated grains could be used for substitution of the wheat flower in the dough. Collectively, our novel findings demonstrated that biochemically defined germination conditions could be used to produce proso millet flour with greater digestibility and nutritive value for the development of new GFD recipes.

Key words: proso millet, germination, enzymatic activity.

INTRODUCTION

During past decade, an incidence of gluten sensitivity diseases and behavioral eating disorders is on the rise worldwide (Fisher et al., 2014; Volta et al., 2017). As diet and nutrition are the primary treatments for these disorders, there is an active search for new sources and means to produce bioavailable foods with pre-defined properties for gluten free diet (GFD). Of particular interest is the development of new technologies utilizing biologically modified millets. Out of multiple species, proso millet (*Panicum miliaceum L.*) is one of the most common cultivated millets worldwide. The growing interest to proso millet is mostly driven by its high nutritive value, high content of calcium, iron, potassium, magnesium, phosphorous, zinc, dietary fiber, protein (Kalinova & Moudry, 2006), B-complex vitamins and sulfur-containing essential amino acids (methioninee and cysteine) (Saleh et al., 2013). Interest to this millet is also dictated by several economically important features: it is relatively drought-tolerant and produces grain with as little as 350 mm of annual rainfall (Oelke et al., 1990). It can be also cultivated in colder climates (up to 54ºN latitude) and in low pH soils (Gulati et al., 2017). Its selective cultivars can produce robust harvests and provide higher yield of flour (77.5−83.7% dry weight) as compared to wheat grains (70−75% dry weight). Currently, most of proso millet is produced in Asia (51.3%) and Africa (44.6%). Europe and Americas produce 3% and 1% of this millet, respectively. In 2017, U.S. farms produced 14.6 million bushels of proso millet for a total crop value of 44.5 million in 2017. Although in US and Europe it is mostly used as a bird and animal feed, proso millet was recently introduced as a whole grain alternative in a healthy gluten-free diet because it contains about 12% of protein, 8% of crude fiber, and 76% of total digestible nutrients (USDA−NASS, 2018). Prior studies demonstrated that germination of proso millet grains increases free amino acids and total sugars and decreased the dry weight and starch content (Parameswaran & Sadasivam, 1994). Considering that germination is associated with enzymatic hydrolysis of various biopolymers and enhanced digestibility (Andriotis et al., 2016; Diaz-Mendoza et al., 2019), in the current study, we conducted a longitudinal biochemical characterization of the germinating proso millet grains. We defined optimal conditions for germination and demonstrated that flour from biologically modified grains can be successfully used for substitution of the wheat flour in non-rising dough-based products.

MATERIALS AND METHODS

Materials

Four cultivars (Alba, Sputnik, Kazak, and Regent) of proso millet (*Panicum miliaceum L.*) were used in the study. For biochemical characterization, grains were germinated in water, 1% NaCl, 1% sucrose and whey.

Assessment of enzymatic activities

To provide basic biochemical characterization, amylolytic, proteinase and lipase activities were measured. Amylolytic activity was assessed by spectrophotometry-based quantitation of the unconverted starch using acidic iodine solution according to standard protocol (Barrera et al., 2016). Proteolytic activity was analyzed by measuring low molecular weight peptides and amino acids after hemoglobin hydrolysis in highly acidic (pH 3.0), low acidic (pH 5.3), neutral (pH 7.0) and highly alkaline (pH 9.0) conditions. After completion of reaction, proteolysis was terminated and non-hydrolyzed hemoglobin was precipitated with trichloroacetic acid. Free amino acids and peptides were then measured by spectrophotometer as described previously (Galleschi et al., 2002). Lipase activity was defined by the speed of the enzymatic hydrolysis of the triacylglycerides of vegetable oil, which was assessed by lipase-mediated release of fatty acids and quantified by titration as described previously (Rose & Pike, 2006). Assessment of cumulative phenols was done by spectrophotometry using Folin-Denis reagent according to standard protocol (Padda & Picha, 2007).

Sensory evaluation

Organoleptic assessment was conducted according to the State Standard #31986−2012 ‘Method of organoleptic assessment of the quality of products for public nutrition′ Standardinform 2014. Briefly, evaluation was done using standard duo-trio test. The testers were given three samples. One testing sample was marked as a reference sample; the other two samples were given coded. The testers determined which sample corresponds to the reference sample and which one is different from it. Baked products sensory profile was described by appearance, color, smell, texture, and taste, which were graded by each taster (scores from 1 to 5 including decimals, with 5 being the highest). Then, average and cumulative scores were calculated. Deviation from the reference sample was evaluated based on t-test.

Statistical analysis

Comparison of changes in enzymatic activities and in phenolic content was done using two-tail t-test. Changes were considered significant when $p < 0.05$. For the sensory evaluation, the average score for each evaluative point was calculated

RESULTS AND DISCUSSION

Germination increases enzymatic activity in proso millet grains

During germination, activation of various enzymes in grains leads to hydrolysis of different biopolymers making them more assessable for digestive system. These enzymes also influence on the quality of the baked products. Thus, amylases are necessary for even crumb structure and a high loaf volume; lipases modify natural lipids to strengthen the dough, and proteases weaken gluten to give plastic properties required in dough for biscuits. To define conditions supporting activation of these enzymatic activities in germinating proso millet, we tested several germinating media including water, 1% NaCl, 1% sucrose and whey. Four different cultivars were germinated for 5 days in these media. With 24 h intervals, samples (100 g) of germinating grains were collected and processed for the assessment of amylolytic, proteinase and lipase activities.

Changes in amylase activity in grains germinated in different media followed similar trend: enzymatic activity was significantly increased by day 3 (Table 1) following a drop to a starting levels within 2 additional days. Germination of grains in sucrose and whey provided the highest activation of the amylolytic activity.

Proteinase and lipase activities in germinating grains were also rising during germination and reached their maximum by day 3. On the contrary to amylase activity, both proteinase and lipase activities sustained at higher levels, albeit some decrease was observed after the 3rd day (Tables 2, 3). Comparison of averages of all cultivars demonstrated that germination in whey provided the highest activation of all enigmatic activities. All observed changes were statistically significant. Besides assessing enzymatic activities, changes in cumulative phenolic content were also evaluated in grains germinated in water. As shown in Table 4, the overall concentration of phenols increased during germination reaching its maximum by day 5. At day 4, the highest level of cumulative phenols was detected in cultivar Sputnik, whereas at day 5 – in Regent and Alba (Table 4). Overall, germination increased phenolic content levels in all cultivars 2–3.5 times within 5 days. These finding are in agreement with prior data showing higher phenolic content in germinating canary seeds and rye grains (Liukkonen et al., 2003).

Table 1. Changes in amylase activity in germinating grains (units ± SD)

Germinating media	Days	Cultivars				*p*-value*
		Sputnik	Regent	Kazak	Alba	
Water	1	1.81 ± 0.01	1.67 ± 0.01	1.69 ± 0.01	1.58 ± 0.01	
	2	2.52 ± 0.01	2.31 ± 0.01	2.46 ± 0.01	1.99 ± 0.01	
	3	2.99 ± 0.01	3.09 ± 0.01	2.99 ± 0.01	2.93 ± 0.01	$p < 0.05$
	4	1.99 ± 0.01	1.48 ± 0.01	1.81 ± 0.01	1.58 ± 0.01	
	5	1.00 ± 0.01	1.29 ± 0.01	1.10 ± 0.01	1.24 ± 0.01	
1% NaCl	1	1.43 ± 0.01	1.67 ± 0.01	1.84 ± 0.01	1.67 ± 0.01	
	2	2.54 ± 0.02	2.52 ± 0.02	2.43 ± 0.02	2.40 ± 0.02	
	3	2.67 ± 0.03	2.66 ± 0.03	2.67 ± 0.03	2.64 ± 0.02	$p < 0.05$
	4	1.75 ± 0.01	1.63 ± 0.01	1.54 ± 0.01	1.69 ± 0.01	
	5	1.34 ± 0.01	1.29 ± 0.01	1.28 ± 0.01	1.28 ± 0.01	
1% Sucrose	1	1.43 ± 0.01	1.64 ± 0.01	1.81 ± 0.01	1.54 ± 0.01	
	2	1.84 ± 0.01	1.87 ± 0.01	2.08 ± 0.02	1.81 ± 0.01	
	3	2.99 ± 0.03	3.05 ± 0.03	2.96 ± 0.03	3.14 ± 0.03	$p < 0.05$
	4	1.66 ± 0.01	1.46 ± 0.01	1.78 ± 0.01	1.58 ± 0.01	
	5	1.08 ± 0.01	1.28 ± 0.01	1.19 ± 0.01	1.37 ± 0.01	
Whey	1	1.49 ± 0.01	1.25 ± 0.01	1.46 ± 0.01	1.28 ± 0.01	
	2	2.52 ± 0.02	2.52 ± 0.02	2.55 ± 0.02	2.05 ± 0.02	
	3	3.05 ± 0.03	3.16 ± 0.03	2.96 ± 0.03	3.31 ± 0.03	$p < 0.05$
	4	1.55 ± 0.01	1.87 ± 0.01	1.66 ± 0.01	1.84 ± 0.01	
	5	1.28 ± 0.01	1.28 ± 0.01	1.31 ± 0.01	1.40 ± 0.01	

*For all cultivars, increase in the enzymatic activity achieves statistical significance ($p < 0.05$) at day 3 as compared to starting point.

Table 2. Changes in protease activity in germinating grains (units ± SD)

Germinating media	Days	Cultivars				*p*-value*
		Sputnik	Regent	Kazak	Alba	
Water	1	0.243 ± 0.017	0.231 ± 0.016	0.206 ± 0.014	0.244 ± 0.017	
	2	0.252 ± 0.018	0.244 ± 0.017	0.230 ± 0.016	0.250 ± 0.018	
	3	0.278 ± 0.019	0.275 ± 0.019	0.290 ± 0.020	0.281 ± 0.020	$p < 0.05$
	4	0.276 ± 0.019	0.235 ± 0.017	0.278 ± 0.019	0.244 ± 0.017	
	5	0.255 ± 0.018	0.220 ± 0.015	0.249 ± 0.017	0.233 ± 0.016	
1% NaCl	1	0.176 ± 0.012	0.199 ± 0.014	0.196 ± 0.014	0.191 ± 0.013	
	2	0.195 ± 0.014	0.235 ± 0.017	0.225 ± 0.016	0.226 ± 0.016	
	3	0.238 ± 0.016	0.258 ± 0.018	0.258 ± 0.018	0.254 ± 0.018	$p < 0.05$
	4	0.229 ± 0.016	0.234 ± 0.016	0.239 ± 0.017	0.245 ± 0.017	
	5	0.220 ± 0.015	0.220 ± 0.015	0.225 ± 0.015	0.233 ± 0.016	
1% Sucrose	1	0.249 ± 0.017	0.246 ± 0.017	0.226 ± 0.016	0.265 ± 0.019	
	2	0.260 ± 0.018	0.268 ± 0.019	0.265 ± 0.019	0.279 ± 0.020	
	3	0.278 ± 0.019	0.273 ± 0.019	0.293 ± 0.020	0.298 ± 0.020	$p < 0.05$
	4	0.268 ± 0.018	0.265 ± 0.019	0.276 ± 0.019	0.280 ± 0.019	
	5	0.251 ± 0.018	0.228 ± 0.016	0.250 ± 0.018	0.265 ± 0.019	
Whey	1	0.253 ± 0.018	0.266 ± 0.019	0.244 ± 0.017	0.274 ± 0.019	
	2	0.269 ± 0.019	0.278 ± 0.019	0.290 ± 0.020	0.304 ± 0.020	
	3	0.279 ± 0.019	0.285 ± 0.020	0.304 ± 0.020	0.320 ± 0.020	$p < 0.05$
	4	0.240 ± 0.017	0.245 ± 0.017	0.284 ± 0.020	0.300 ± 0.020	
	5	0.235 ± 0.016	0.231 ± 0.016	0.256 ± 0.018	0.260 ± 0.018	

*For all cultivars, increase in the enzymatic activity achieves statistical significance ($p < 0.05$) at day 3 as compared to starting point.

Our data showing an increased enzymatic activities in germinating grains supports prior studies showing that germination is associated with high mineral content of foxtail millet (Coulibaly, 2011) and with increased anti-oxidant activity of the finger millet (*Eleusina coracana*) (Abioye et al., 2018). Moreover, our studies demonstrated that germination for 3 days is optimal for the induction of the enzymes and that longer germination reduces proteolytic, amylase, and lipase activities. The importance of these novel findings is emphasized by fact that these enzymes increase bioactivity, nutritional value, and the overall quality of baked products. Of particular interest to further development of the technology is the observed increase in cumulative phenolic content in germinated proso millet grains.

Table 3. Changes in lipase activity in germinating grains (units ± SD)

Germinating media	Days	Cultivars				*p* -value*
		Sputnik	Regent	Kazak	Alba	
Water	1	0.40 ± 0.02	0.53 ± 0.02	0.38 ± 0.02	0.42 ± 0.02	$p < 0.05$
	2	0.45 ± 0.02	0.60 ± 0.02	0.42 ± 0.02	0.45 ± 0.02	
	3	0.90 ± 0.03	1.04 ± 0.03	0.86 ± 0.03	0.80 ± 0.02	
	4	0.95 ± 0.03	1.02 ± 0.03	0.86 ± 0.03	0.76 ± 0.03	
	5	0.80 ± 0.03	0.90 ± 0.03	0.80 ± 0.03	0.60 ± 0.02	
1% NaCl	1	0.44 ± 0.02	0.55 ± 0.02	0.40 ± 0.02	0.43 ± 0.02	$p < 0.05$
	2	051 ± 0.02	0.62 ± 0.02	0.46 ± 0.02	0.47 ± 0.02	
	3	1.08 ± 0.03	0.96 ± 0.03	0.88 ± 0.03	0.86 ± 0.03	
	4	1.02 ± 0.03	0.92 ± 0.03	0.82 ± 0.03	0.80 ± 0.03	
	5	0.82 ± 0.03	0.90 ± 0.03	0.75 ± 0.03	0.70 ± 0.03	
1% Sucrose	1	0.38 ± 0.02	0.48 ± 0.02	0.39 ± 0.02	0.40 ± 0.02	$p < 0.05$
	2	0.40 ± 0.02	0.51 ± 0.02	0.41 ± 0.02	0.42 ± 0.02	
	3	0.84 ± 0.03	0.78 ± 0.03	0.30 ± 0.03	0.77 ± 0.03	
	4	0.80 ± 0.03	0.75 ± 0.02	0.78 ± 0.02	0.75 ± 0.02	
	5	0.67 ± 0.02	0.60 ± 0.02	0.68 ± 0.02	0.66 ± 0.02	
Whey	1	0.46 ± 0.02	0.51 ± 0.02	0.40 ± 0.02	0.42 ± 0.02	$p < 0.05$
	2	0.52 ± 0.02	0.56 ± 0.02	0.47 ± 0.02	0.45 ± 0.02	
	3	1.16 ± 0.03	1.12 ± 0.03	0.92 ± 0.03	0.84 ± 0.03	
	4	1.10 ± 0.03	1.08 ± 0.03	0.90 ± 0.03	0.80 ± 0.03	
	5	0.98 ± 0.03	0.96 ± 0.03	0.72 ± 0.02	0.72 ± 0.02	

*For all cultivars, increase in the enzymatic activity achieves statistical significance ($p < 0.05$) at day 3 as compared to starting point.

Table 4. Changes in cumulative phenols in germinating grains

Day of germination	Cumulative phenols in cultivars (mg kg^{-1})				*p*-value*
	Sputnik	Kazak	Regent	Alba	
Dry grain	525 ± 7	550 ± 7	575 ± 8	525 ± 6	$p < 0.05$
1	425 ± 6	462 ± 6	545 ± 8	450 ± 5	
2	460 ± 6	470 ± 7	562 ± 8	500 ± 5	
3	1,550 ± 17	1,025 ± 11	775 ± 7	700 ± 6	
4	1,700 ± 18	1,125 ± 12	950 ± 12	850 ± 7	
5	1,250 ± 13	1,145 ± 12	1,850 ± 19	1,820 ± 19	

*For all cultivars, increase in cumulative phenols achieves statistical significance ($p < 0.05$) at day 3 as compared to starting point.

Polyphenols are important antioxidants (Kadiri, 2017), which at low concentration can delay or prevent oxidation of an oxidizable substrates and could reduce risk of cardiovascular disease and cancer (Kris-Etherton et al., 2002). They can also mitigate oxidative stress on DNA. Because amounts of cumulative phenols in germinating proso millet (up to 1,850 μg g^{-1}) are compatible and even higher than in bran layer (1,258–3,157 μg g^{-1}) and whole wheat grains (168–459 μg g^{-1}) (Vaher et al., 2010), flour from germinating proso millet could be used in design of selective diets with high antioxidant capacity.

Flour from germinating proso millet grains could be used in non-rising dough for wheat flower substitution.

Based on the obtained data, we produce a batch of flour from Sputnik and Regent grains germinated in water for 3 days. Germination in water was selected because it showed a median rise of all enzymatic activities and cumulative phenolic content, as compared to other media. The resultant flour was used for partial or complete substitution of the wheat flour in non-rising cake and shortbread dough. Following preparation, the final products were assessed and graded by 10 independent tasters for visual appearance, color, smell, texture, and taste. Products prepared from the wheat flour according to the same recipe were used as a reference control. Based on this evaluation, all shortbread pastry with 30% and 50% substitution of wheat flour did not substantially deviate from control samples and were graded similarly to the wheat flour-based products. Cakes will 50% substitution also did not substantially deviate from wheat-based products. Despite lower scores for several parameters, all products, particularly shortbreads, prepared from 100% substituted flour, were evaluated as compatible with wheat flour-based products (Table 5).

Table 5. Sensory assessment results of partial or complete substitution of the wheat flour in non-rising cake and shortbread dough

Assessed	Ratio of wheat/germinated proso millet flower in cake // shortbread flour			
parameters	100/0	70/30*	50/50	0/100
Appearance	5.0	// 4.8	4.0 // 5.0	4.0 // 3.8
Color	5.0	// 4.5	4.7 // 4.9	4.3 // 4.9
Smell	5.0		4.8 // 4.8	4.0 // 4.2
Taste	5.0	// 4.8	4.8 // 4.9	4.5 // 4.6
Structure	5.0	// 4.7	4.7 // 4.9	4.3 // 4.0
Combined Score*	25.0	// 23.5	23.9 // 24.5	21.7 // 21.5

* 70/30 ratio was used only for shortbread flour; ** all scores reflect deviations from the control sample and not the quality of the product; presented scores are the averages of scores from all testers.

The utility of biologically modified millet flours in traditional millet-based foods (e.g. instant fura) were previously tested (Amadou et al., 2011). In this study, besides providing biochemical characterization, we also test the utility of the flour from germinating proso millet for partial and complete substitution of the wheat flower in traditionally wheat-based non-rising dough products. Our evaluation confirmed that this flour could be successfully used for partial replacement of wheat flour in a cake and shortbread dough and complete replacement – in a cake dough without substantial change in basic sensory characteristics. Out of all, typical wheat flour smell was missing in products with 100% substitution and products crumbled easily. Yet, these drawbacks did not reduce the overall appeal of the baked products, particularly shortbread pastries.

CONCLUSIONS

Collectively, our studies demonstrated that germination of proso millet grains for 3 days is optimal for activation of enzymatic activities to increase bioavailability of the flour which could be successfully used for partial and complete substitution of wheat flour in wheat flour-based recipes. Our findings provide the basis for the further development of the technology for the preparation of healthy and nutritional food products such as complementary food products, composite flours, GFD products, and infant formula.

REFERENCES

Abioye, V., Ogunlakin, G., Taiwo, G.A. 2018. Effect of Germination on Anti-oxidant Activity, Total Phenols, Flavonoids and Anti-nutritional Content of Finger Millet Flour. *Journal of Food Processing & Technology* **9**, 719.

Amadou, I., Le, G.-W., Gbadamosi, O.S. 2011. Millet-based raditional Processed Foods and Beverages − A Review. *Sereal Food World* **56**, 115−121.

Andriotis, V.M., Rejzek, M., Rugen, M.D., Svensson, B., Smith, A.M. & Field, R.A. 2016. Iminosugar inhibitors of carbohydrate-active enzymes that underpin cereal grain germination and endosperm metabolism. *Biochem. Soc. Trans.* **44**, 159−165.

Barrera, G.N., Tadini, C.C., Leon, A.E. & Ribotta, P.D. 2016. Use of alpha-amylase and amyloglucosidase combinations to minimize the bread quality problems caused by high levels of damaged starch. *J. Food Sci. Technol.* **53**, 3675−3684.

Coulibaly, C. 2011. Evolution of Energetic Compounds, Antioxidant Capacity, Some Vitamins and Minerals, Phytase and Amylase Activity During the Germination of Foxtail Millet. *American Journal of Food Technology* **6**, 40−51.

Diaz-Mendoza, M., Diaz, I. & Martinez, M. 2019. Insights on the Proteases Involved in Barley and Wheat Grain Germination. *Int. J. Mol. Sci.* **20**.

Fisher, M.M., Rosen, D.S., Ornstein, R.M., Mammel, K.A., Katzman, D.K., Rome, E.S., Callahan, S.T., Malizio, J., Kearney, S. & Walsh, B.T. 2014. Characteristics of avoidant/restrictive food intake disorder in children and adolescents: a ‘new disorder’ in DSM-5. *J. Adolesc. Health.* **55**, 49−52.

Galleschi, L., Capocchi, A., Ghiringhelli, S., Saviozzi, F., Calucci, L., Pinzino, C, & Zandomeneghi, M. 2002. Antioxidants, free radicals, storage proteins, and proteolytic activities in wheat (Triticum durum) seeds during accelerated aging. *J. Agric. Food Chem.* **50**, 5450−5457.

Gulati, P., Li, A., Holding, D., Santra, D., Zhang, Y. & Rose, D.J. 2017. Heating Reduces Proso Millet Protein Digestibility via Formation of Hydrophobic Aggregates. *J. Agric. Food Chem.* **65**, 1952−1959.

Kadiri, O. 2017. A review on the status of the phenolic compounds and antioxidant capacity of the flour: Effects of cereal processing. *International Journal of Food Properties* **20**, S798−S809.

Kalinova, J. & Moudry, J. 2006. Content and quality of protein in proso millet (Panicum miliaceum L.) varieties. *Plant Foods. Hum. Nutr.* **61**, 45−49.

Kris-Etherton, P.M., Hecker, K.D., Bonanome, A., Coval, S.M., Binkoski, A.E., Hilpert, K.F., Griel, A.E. & Etherton, T.D. 2002. Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. *Am. J. Med.* **113**(Suppl 9B), 71S−88S.

Liukkonen, K.-H., Katina, K., Wilhelmsson, A., Myllymaki, O., Lampi, A.-M., Kariluoto, S., Piironen, V., Heinonen, S.-M., Nurmi, T., Adlercreutz, H., Peltoketo, A., Pihlava, J.-M., Hietaniemi, .-M. & K. Poutanen. 2003. Process-induced changes on bioactive compounds in whole grain rye. *Proceedings of the Nutrition Society* **62**, 117−122.

Oelke, E.A., Oplinger, E.S., Putnam, D.H.,Durgan, B.R., Doll, J.D. & Undersander, D.J. 1990. Millets:Alternative Field Crop Manual. CAPAP and the Minnesota Extension Service.

Padda, M.S. & Picha, D.H. 2007. Methodology Optimization for Quantification of Total Phenolics and Individual Phenolic Acids in Sweetpotato (Ipomoea batatas L.) Roots. *Journal of Food Science* **72**, C412−C416.

Parameswaran, K.P. & Sadasivam, S. 1994. Changes in the carbohydrates and nitrogenous components during germination of proso millet, Panicum miliaceum. *Plant. Foods. Hum. Nutr.* **45**, 97−102.

Rose, D.J. & Pike, O.A. 2006. A simple method to measure lipase activity in wheat and wheat bran as an estimation of storage quality. *Journal of the American Oil Chemists' Society* **83**, 415−419.

Saleh, A.S., Zhang, Q.,Chen, J. & Andshen, Q. 2013. Milletgrains:nutritional quality, processing, and potential health benefits. *Comprehensive Reviews in Food Science and Food Safaty* **12**, 218−295.

Vaher, M., Matso, K., Levandi, T., Helmja, K. & Kaljurand, M. 2010. Phenolic compounds and the antioxidant activity of the bran, flour and whole grain of different wheat varieties. *Procedia Chemistry* **2**, 76−82.

Volta, U., Caio, G., Karunaratne, T.B., Alaedini, A. & De Giorgio, R. 2017. Non-coeliac gluten/wheat sensitivity: advances in knowledge and relevant questions. *Expert Rev Gastroenterol Hepatol* **11**, 9−18.

Agronomy Research **17**(6), 2195–2202, 2019
https://doi.org/10.15159/AR.19.223

Optimization of plant densities of dolichos (*dolichos lablab* L. var. *lignosus*) bean in the Right-bank of Forest-steppe of Ukraine

I. Bobos[1], I. Fedosy[1], O. Zavadska[1], O. Tonha[1] and J. Olt[2,*]

[1]National University of Life and Environmental Sciences of Ukraine, Heroyiv Oborony 15, UA03041 Kyiv, Ukraine
[2]Estonian University of Life Sciences, Institute of Technology, Kreutzwaldi 56, EE51006 Tartu, Estonia
[*]Correspondence: jyri.olt@emu.ee

Abstract. The density of the plants of Dolichos bean significantly influenced the economically valuable indicators, because there is always competition for light, moisture and nutrients between plants in the life process. The period from mass sprouting to the technical ripeness was reduced with increasing the plant density. Such a pattern was characteristic of all phases of the growth and development of the Dolichos bean. The plants with high population (71 thousand units ha^{-1}) took short period (60 and 119 days) from germination to the beginning of technical and biological ripeness, respectively, turned out to be the earliest ripening crops. The plants are better illuminated, the soil nutrition conditions are improved and the sanitary-hygienic climate of the crops improves with thinned crops, thereby plant productivity has raised. However, the average yield of scapulabeans and unripe Dolichos seeds is regulated by the density of the plants, and increased in density due to the greater number of plants. The optimum density for Dolichos bean was 71 thousand plants per hectare, at which yields of green shoots and green peas were formed 7.3 and 3.3 t ha^{-1}, respectively.

Key words: beans scapula, dolichos bean, ripening beans, plant density, sowing scheme, stairs, flowering, yield, unripe seeds.

INTRODUCTION

Currently, there are many unsolved problems in the vegetable growing industry, such as, for example, the insufficient species diversity in the range of vegetable crops, the low yield and quality levels in the vegetable production. The issues of providing the population with food rich in protein, which is lacking in the daily diet of each person, are also considered. Very valuable legumes, as important and cheap source of protein, which the poor modern human diet is short of (Chattopadhyay & Dutta, 2010; Upadhyay & Mehta, 2010; Chauhan et al., 2012; Sych & Bobos 2013; Bajwa, 2014; Habib et al., 2017; Arul Ananth & Ramesh Kumar, 2018; Ranaivoson et al., 2019), are among them.

In Ukraine, vegetable farming has now become an attractive business. In recent years, positive results were achieved in the improvement of the yield, the total output, the quality of the vegetables and the volume of exports. At the same time, the analysis of the current situation in the production of vegetables in farm units of different forms of ownership and the marketing trends gives evidence of the emergence of new challenges, which hamper the positive development of this branch of agriculture. Theseinclude first of all excessive production and strong competition around a very narrow range of vegetable crops. The whole industry relies on the borsch (beetroot and cabbage soup) group of vegetables, tomatoes, cucumbers and some other crops.

Among the main ways of overcoming the mentioned problems, the expansion of the production range is the most promising one. Moreover, this line of development implies increasing not only the number of varieties, but the number of species as well.

The food crop species of the legume family are part of the range of the plants that are currently known and agriculturally promising, but have not yet gained wide recognition in the market.

Among the large legume family, there is one very interesting genus – dolichos (*Dolichos* L.) (Joshi & Rahevar, 2015). Among 60 species, far to the north, only one species has spread – dolichos bean, or hyacinth beans (*Dolichos lablab L*). In the southern countries it is valued for its medicinal properties and edible seed, in Europe it is decorative. Decorative beautiful red leaves and flowers, resembling orchids, with the scent of hyacinth. Beautiful beetroot-colored beans have a parchment layer, so only unripe (type flageoles) and ripe black seeds can be used as food, which is considered an important medicinal raw material for dissolving kidney stones (Bobos, 2016; Bobos & Ivanitskaya, 2018).

The yield of Dolichos bean, like of other crops, is formed in specific soil and climatic conditions of cultivation and is the result of the reaction of plants to them. However, the development of plants is influenced not only by soil and climatic conditions, but also by technological interventions.

Among the main technological measures aimed at increasing yields, an important role belongs to the choice of scientifically based seeding rate and plant density of Dolichos in crops, with the help of which optimal areas of plant nutrition are created. At the choice of the optimal plant density of Dolichos bean there are no scientific and theoretical studies. All this leads to the need to study and establish the most rational feeding areas for the Dolichos plants in the Right-Bank Forest-Steppe of Ukraine, in which optimal conditions for the growth and development of plants and yield formation (Sych & Bobos, 2013) will be created.

The aim of the research was to identify the adaptive properties of Dolichos bean based on the study of the effect of plant density on the yield of scapula beans to obtain unripe seeds in the conditions of the Kyiv region.

MATERIALS AND METHODS

Studies were conducted in 2013–2015 at the collection site of the Department of Vegetable and Closed Ground in the SRI ‘Fruit and Vegetable Garden’ of the National University of Life and Environmental Sciences of Ukraine in three replications according to the method of single-factor experiments (Bondarenko & Yakovenko, 2001). The subject of research was the specious of dolichos (*Dolichos lablab L*). Sowing schemes: 70×20 (71 thousand units ha^{-1}), 70×30 (48 thousand units ha^{-1}), 70×40 (36 thousand units ha^{-1}), 70×50 (29 thousand) units/ha) were studied. A seeding scheme of 70×40 cm was taken for control. Dolichos bean was grown as per Sych (2010).

The seeds were sown simultaneously (2013–10.05, 2014–07.05, 2015–02.05). The depth of seeding was 2–3 cm. The size of the land area was 5 m^2. The feeding area was regulated by the number of plants in a row.

The research into the growing space for dolichos was carried out using the method of single-factor experiments on humic mesopodzol soil. The object of research was the local cultivar of dolichos selected during the previous investigations at the Vegetable Farming Department, in which the following planting systems had been applied: 70×20, 70×30, 70×40, 70×50 cm. The number of replications was three, with randomization. The recorded plot area was 5 m^2. The records were made for 30 plants – 10 from each replication. Phenological observations, biometric measurements, bean and seed yield accounting and biochemical content of seeds were performed. The results were processed by a statistical program 'Agrostat'.

The obtained data are reflected in the results of the research.

The soil in the plot was dark grey light loam mesopodzol. The humic layer thickness was 24–28 cm.

The experimental plot featured a low humus content of 1.5–2.2% and medium contents of hydrolysable nitrogen - 26–38 mg kg^{-1}, labile phosphorus - 43–61 mg kg^{-1} and potassium - 28–34 mg kg^{-1}.

In the crop rotation system, dolichos succeeded cucumbers. The basic soil preparation for the crop included the autumn operations on the removal of the plant debris from the preceding crop and weeds and deep ploughing. The plot was disked to a depth of 6-8 cm with an LDG-10A disk stubble cleaner aggregated with a T-150 tractor moving in two directions. The autumn ploughing was performed in the second ten-day period of September to a depth of 25–27 cm with the use of a PLN-5-35 plough with coulter aggregated with a T-150 tractor. The plot was harrowed early in the spring diagonally to the line of ploughing with the use of BZTS-1.0 toothed harrows aggregated with an SP-16 multiple hitch and a T-150 tractor. In the second to third ten-day periods of April and prior to seeding, the experimental plot was cultivated to depths of 12–15 cm and 6–8 cm, respectively, with the use of a KPSP-4 cultivator aggregated with a DT-75 tractor and levelled off with the use of a harrow, then the dolichos seeds were drilled.

RESULTS

The results of research proved that the growth and development of plants in the initial stagesoccurred almost simultaneously, the difference in the rates of occurrence of phenological phases was observed within the limits of experimental error – 2–3 days. The emergence of friendly seedlings is often a decisive factor of high yields of agricultural crops. In our experience, the evaluation of the field germination of Dolichos showed that it was almost the same regardless of the density and on average was getting 84%.

Phenological observations of the growth and development of Dolichos plants for different densities were carried out from the emergence of seedlings to the biological ripeness of beans (Table 1). The results of the research revealed that the seeding schemes affected the early maturity of the species. At the same time, dolichos was characterized as a late-ripening species regardless of the seeding scheme. This is due to the extended period of flowering and fruiting species. However, with the high plant density

(71 thousand units ha^{-1}), the vegetation period is shortened by 5 days compared to sparse crops (29 thousand units ha^{-1}) when the species is sown on July 16.

Table 1. The results of phenological observations of the growth and development of plants Dolichos for different densities (average for 2013–2015)

Experimental options	Plant density, ths.pcs·ha^{-1}	Beginning of seed germination (germination start) (10%)	Full shoots (75%)	Beginning of flowering	Beginning of technical maturation of beans	Beginning of the biological maturation of beans
70×20	71	13.05	17.05	24.06	11.07	08.09
70×30	48	14.05	17.05	25.06	14.07	11.09
70×40 (control)	35	16.05	19.05	26.06	14.07	12.09
70×50	29	16.05	19.05	28.06	16.07	15.09

Rather, full shoots appeared in species with thickening (48–71 thousand units ha^{-1}). This is due to the high amount of active temperatures during this period. In sparse crops, full sprouting in species was longer. Low temperatures at the end of May 2014 affected the later appearance of both single and mass shoots.

Weather conditions in May 2014, namely, sharp changes in temperature during the day and night caused later flowering and technical ripeness of the scapula beans for different crop density. However, in the future, the hot weather in July and August 2014 caused rather the passage of technical ripeness of the beans and their biological ripeness compared to 2013 and 2015 years.

The beginning of flowering in this species later was observed for plant densities of 29 thousand units ha^{-1} – 29.06. Moreover, with the thickening of plants, the beginning of flowering and ripening of the beans was noted in 3 days earlier compared with the control. The same trend was observed in species of other seeding schemes.

Table 2. Duration of phenological phases in Dolichos plants by different sowing dates (average for 2013–2015)

Experimental options	Plant density, ths pcs ha^{-1}	Duration of periods, days			
		‘sowing - full shoots’	‘full shoots - the beginning of flowering’	‘full shoots, the beginning of the technical ripeness of the beans’	‘full seedlings, the beginning of the biological ripeness of the beans’
70×20	71	10	44	60	119
70×30	48	11	44	62	123
70×40 (control)	35	12	45	61	123
70×50	29	13	45	64	126

Flowering and fruiting in Dolichos took place during the whole growing season. The duration of the interphase periods of the species was different and depended on the seeding scheme (Table 2). Thus, the duration of the ‘sowing-full seedlings’ period turned out to be the smallest at the highest density itself (48–71 thousand units ha^{-1}) - 10–11 days. This is due to the increased temperature of the soil during the germination of thickened crops, which affected the rapid emergence of plants. A little longer, the duration of this phase was noted in species with lower plant density (29–35 thousand units ha^{-1}) – 12–13 days. Moreover, in 2014, a sharp amplitude of temperature fluctuations, day and night in May, affected the long duration of this period in varying the density of crops.

Unripe scapula beans in the technical ripeness phase have a very beautiful burgundy color. Dolichos is caracterized by the long flowering of beautiful purple flowers from June to autumn frosts. All of this indicates the possibility of its use in landscaping (Fig. 1).

Figure 1. Mass flowering plants of Dolichos for plant density of 71 thousand units·ha^{-1}.

The duration of the period of 'full sprouts-the beginning of the technical ripeness of the beans' is less found in species with the thickening of crops (71 thousand units ha^{-1}), which was 60 days. A trend has been established, with the increase in plant density, the period from flowering to the beginning of the technical ripeness of the beans has decreased. The density of plants also influenced the duration of the period of 'full sprouting – the beginning of the biological ripeness of the beans', which turned out to be less than 70×20 seeding schemes (119 days). This is due to the rapid warming of the soil surface along the greater density of plants, accelerates the beginning of all phases of the growth and development of Dolichos plants.

The beans on the plant do not ripen at the same time, in result the multiple harvesting of scapula beans. The longest period of the formation of beans turned out to be at the lowest plant density (29 thousand units ha^{-1}) – 129 days.

Thus, the density of plants of Dolichos influenced the duration of the interphase periods. Unequal growing conditions consisting in a cenosis of different density are expressed by the duration of the growing season of plants. When increased the plant density, the period from mass sprouting to the technical ripeness was reduced. Such a pattern was characteristic of all phases of the growth and development of the Dolichos plants. The plants of the crop with the thickening of crops (71 thousand units ha^{-1}) with a short duration of the growing season of 60 days were the earliest ripening crops.

It is established that the density of plants of Dolichos significantly influenced the formation of above-ground mass. The experimental data obtained show that the smallest height of the Dolichos plants was for density 71 thousand units ha^{-1}, and with a decrease in density, it grew. The results of the research indicate that the plants of Dolichos are very sensitive to changes in the area of nutrition. Thickened crops affected the number of shoots in plants (Table 3).

Table 3. Characteristics of the morphological characteristics of plants of Dolichos with different sowing patterns (average for 2013–2015)

Experimental options	Plant density, ths pcs ha^{-1}	Stem length, cm	The number of shoots, pieces	The thickness of the stem at the root collar, mm
70×20	71	153.9 ± 8.2	6.6 ± 0.6	12.7 ± 1.3
70×30	48	163.3 ± 7.2	7.0 ± ±0.5	14.3 ± 1.1
70×40 (control)	35	165.9 ± 6.1	7.3 ± 0.7	15.0 ± 1.2
70×50	29	168.4 ± 5.9	8.2 ± 0.9	16.7 ± 1.5

In the process of analyzing the biometric parameters of plants of Dolichos for different density, it should be noted that plants had the most developed vegetative mass at the lowest plant density (29 thousand units ha^{-1}). Moreover, the results of the confidence interval prove that according to all the morphological characters that were studied there is a significant difference between the plants with the lowest and the highest density. Thus, a significantly greater height was found in plants – 168.4 cm, which is in 14.5 cm more compared to the control. With thickening in species, the height of plants decreased. The same trend was observed in other morphological indicators in Dolichos.

The thickening significantly suppressed the optimal growth of the Dolichos plants. This is due to the unlimited type of growth of crop in favorable growing conditions. Therefore, the thickening accompained the less intensive growth of leguminous plants, incl. Dolichos (Sych & Bobos, 2011; Bobos, 2016).

The results show that different plant densities significantly influenced the biometric indicators, because in the living process between plants there is always competition for light, moisture and nutrients. A more developed vegetative mass was characterized by Dolichos plants for sparse crops. To get the Dolichos scapula beans in open ground without supports, thickened crops (71 thousand units ha^{-1}) were more suitable, in which the length of the stem and the number of shoots were respectively 153.9 cm and 6.6 units. with a stalk thickness at the root collar of 12.7 mm.

Table 4. The yield of commercial scapula beans of Dolichos depending on plant density, thousand units / ha (average for 2013–2015)

Experimental options	Plant density, ths. pcs ha^{-1}	Weight of beans from a plant, gr	Yield of scapula beans, t ha^{-1}			The average yield of beans for three years, t ha^{-1}	Yield increase	
			2013	2014	2015		t ha^{-1}	%
70×20	71	102.4				7.3	+2.4	+49
70×30	48	116.3	2.7	3.8	10.1	5.5	+0.6	+12
70×40 (control)	36	137.3	2.9	3.5	8.4	4.9	0	100
70×50	29	145.9	2.4	3.0	7.3	4.8	-0.1	-2
HIP_{05}		10.7	0.4	0.3	0.8	2.2		

The productivity of the Dolichos plants influenced their average productivity of scapula beans (Table 4). The beans ripened in crops not at the same time with different thicknesses, so the harvest was carried out weekly. The highest mass of beans from the plant was got in Dolichos at the lowest density of 29 thousand units ha^{-1}, amounted to 145.9 g, which is in 43.5 g more compared to the control. A lower difference between the controls was revealed in species of the greatest density of 71 thousand units ha^{-1}, which amounted to 34.9 g. At the same time, the average yield of the Dolichos beans for this density was higher to 7.9 t ha^{-1}. This is due to the large number of plants per unit of area.

Figure 2. Simultaneous flowering and fruiting plants of Dolichos.

The lowest yield level was obtained for plant density of 29 thousand units ha^{-1} – 4.8 t ha^{-1}. This can be explained by the smaller number of plants per unit of area, despite the fact that the average mass of beans from a single plant of this variant was the greatest.

Dolichos is marked by a long flowering of beautiful purple flowers from July to autumn frosts and simultaneous fruiting (Fig. 2). All this testifies to the possibility of its use in landscaping (Sych & Bobos 2013; Bobos, 2016; Bobos & Ivanitsky, 2018).

Due to the greatest density (71 thousand units ha^{-1}), the plants were characterized by a higher intensity of early yield (Table 5). Moreover, significantly lower yields of unripe seeds were obtained at the lowest density (29 thousand units ha^{-1}) and amounted to 2.1 t ha^{-1}, which is in 1.2 t ha^{-1} less compared to the control. This is due to the smaller number of plants per unit of area and, accordingly, a smaller number of scapula beans in which unripe seeds were formed.

Table 5. The yield of green peas of Dolichos depending on plant density, thousand units·ha^{-1} (average for 2013–2015)

Experimental options	Plant density, ths pcs ha^{-1}	Yield of green peas, t ha^{-1}			Average yield of green peas, t ha^{-1}	Yield increase	
		2013	2014	2015		t ha^{-1}	%
70×20	71				3.3	+0.7	+27
70×30	48	1.7	2.3	4.6	2.9	+0.3	+12
70×40 (control)	36	1.8	2.1	4.0	2.6	0	100
70×50	29	1.5	1.8	2.9	2.1	-1.2	-19
HIP_{05}		0.2	0.7	0.9	0.6		

Plant density 29–48 thousand units·ha^{-1}favoured to the intensive growth of plants compared to a higher density, which influenced the formation of a larger number of scapula beans during the growing season (Table 5). However, such density did not predetermine a high yield of scapula beans and unripe seeds, therefore it is not suitable for growing of Dolichos without supports in open ground in the Forest-Steppe of Ukraine.

CONCLUSIONS

To enrich the protein diversity of the population, cultivation in the right-bank of Forest-Steppe of Ukraine of Dolichos bean with plant density of 71 thousand units ha^{-1}, at which the highest yield obtained in scapula beans (7.3 t ha^{-1}) and green peas (3.3 t ha^{-1}) is perspective now-a-days.

REFERENCES

Arul Ananth, R. & Ramesh Kumar, S. 2018. Screening of dolichosbean (lablab purpureus L. (Sweet) genotypes for growth and yield in coastal region of Tamilnadu. *Plant Archives* **18**(2), 1258–1262.

Bajwa, A.A. 2014. Sustainable weed management in conservation agriculture. *Crop Protection* **65**, 105–113. doi: 10.1016/j.cropro.2014.07.014

Bobos, I.M. & Ivanitsky, A.R. 2018. Qualitative estimation of beans and seeds of dolichos. Modern Scientific Researches, Agriculture (Yolnat PE, Minsk), **4**, 38–40. Available: https://www.sworld.education/msr/msr4-1.pdf.

Bobos, I.M. 2016. The growth and development of dolichos plants in the Right-bank of Forest-Steppe of Ukraine. SWorld Journal "Scientific world". Ivanovo, Vol. **J116**(10). Available: http://www.sworld.education/e-journal/j11609.pdf

Bondarenko, G.L., Yakovenko K.I. 2001. *Methodology of Experimental Case in Vegetable and Melons*. Ed.. Kharkiv, Osnova, 369 pp.

Chattopadhyay, A. & Dutta, S. 2010. Characterization and identification of selection indices of pole type dolichos bean. *Vegetable Crops Research Bulletin* **73**(1), 33–45. doi: 10.2478/v10032-010-0016-9

Chauhan, B.S., Singh, R.G. & Mahajan, G. 2012. Ecology and management of weeds under conservation agriculture: A review. *Crop Protection* **38**, 57–65. doi: 10.1016/j.cropro.2012.03.010

Habib, H.M., Theuri, S.W., Kheadr, E.E. & Mohamed, F.E. 2017. Functional, bioactive, biochemical, and physicochemical properties of the Dolichos lablab bean. *Food and Function* **8**(2), 872–880. doi: 10.1039/c6fo01162d

Joshi, S.K. & Rahevar, H. 2015. Effect of dates of sowing, row spacings and varieties on growth and yield attributes of rabi Indian bean (Golichos lablab L.). *Indian Journal of Agricultural Research* **49**(1), 59–64. doi: 10.5958/0976-058X.2015.00008.6

Methodology of Experimental Case in Vegetable and Melons. 2001. Ed. G.L. Bondarenko, K.I., Yakovenko. Kharkiv, Osnova, 369 pp.

Ranaivoson, L., Naudin, K., Ripoche, A., Rabeharisoa, L. & Corbeels, M. 2019. Effectiveness of conservation agriculture in increasing crop productivity in low-input rainfed rice cropping systems under humid subtropical climate. *Field Crops Research* **239**, 104–113. doi: 10.1016/j.fcr.2019.05.02

Sych, Z.D. & Bobos, I.M. 2013. The new vegetable plants are in the modern vegetable business. Earth Bioresources and Quality of Life. International Electronic Journal of Science. Available: http://gchera-ejournal.nubip.edu.ua

Sych, Z.D. 2010. Recommendations for the growing of leguminous vegetable crops in the forest-steppe Ukraine. Kyiv, NULES of Ukraine, 41 pp.

Upadhyay, D. & Mehta, N. 2010. Biomedical studies in Dolichos Bean (Dolichos lablab L.) for Chattisgarh Plains. *Res. J. Agri. Sci*. **1**(4), 441–447.

Agronomy Research **17**(6), 2203–2210, 2019
https://doi.org/10.15159/AR.19.200

Comparison of a 1 t and a 55 t container when storing spelt grain in mild climate of the Czech Republic

J. Bradna[1,*], J. Šimon[1], D. Hájek[1], D. Vejchar[1], I. Polišenská[2] and I. Sedláčková[2]

[1]Research Institute of Agricultural Engineering, p. r. i., Drnovská 507, CZ161 01 Prague 6 - Ruzyně, Czech Republic
[2]Agrotest fyto, Ltd., Havlíčkova 2787/121, CZ767 01 Kroměříž, Czech Republic
[*]Correspondence: jiri.bradna@vuzt.cz

Abstract. Maintaining a suitable microclimate inside the storage space is the most significant factor in maintaining good quality of stored grain for small farmers. This article is aimed at evaluating the influence of outdoor climatic conditions on the storage conditions, specifically the temperature of stored grain in two storage containers. One structure was a 4×6 m cylindrical container (55 t capacity) with a steel wire mesh wall lined with a textile shell. Spelt grain (*Triticum spelta*) was also stored simultaneously at the same location in a fabric intermediate bulk container (FIBC) bag with maximum capacity of 1 t. Neither structure was mechanically aerated. Grain moisture and temperature were monitored during the spring and start of the summer period of the year 2017 because of the biggest differences between the night and day temperatures. For monitoring of the grain microbiological changes samples were taken for laboratory tests during the whole experiment. Grain quality parameters measured during storage included the bulk density, crude protein, falling number, germination, gluten content, sedimentation index and contamination by mycotoxins. Monitored outdoor environment parameters were temperature, dew point and relative humidity. Results showed a strong dependence of the stored material temperature on the outside temperature in the case of FIBC bags (coefficient of determination $R^2 = 0.927$), whereas the dependence was weaker in the larger structure ($R^2 = 0.625$). Mycotoxins monitored during the period were below the detection limit in both cases.

Key words: postharvest preservation, DON, ochratoxin A, grain quality factors, storage conditions, temperature.

INTRODUCTION

During post-harvest treatment and storage, the mechanical properties of individual food grain kernels have many opportunities to be damaged when subjected to stresses after falling from various heights on its traffic route and finally into the storage container. There is a direct correlation between grain damage during post-harvest treatment and transport, i.e. the content of grain fragments, and the time the cereal can be safely stored (Bradna et al., 2018). A question is also the quantification of changes in some qualitative parameters such as germination during storage. There have been statistically significant correlations between some physical parameters and mycotoxins (Capouchová et al., 2009).

Grain moisture and storage temperature are the basic parameters in maintaining the correct microclimate throughout the storage period. According to Hammami et al. (2017), high moisture content in food wheat affects increased respiration rate due to enzymatic activity and also faster development of moulds and their products (mycotoxins). These negative factors have a significant influence on the final quality of the grain at the end of the storage period and, therefore, on the final market potential and price of each material lot (Hammami et al., 2017). The moisture content of spelt wheat for storage is not bound by Czech legislation. Based on the legal framework for further processing of grains for food purposes (Czech Decree No. 333/1997 Sb.) and for seed purposes (Czech Decree No. 129/2012 Sb.), the maximum value of grain moisture can be set at 15% wt. The same value was also reported by Zuk-Gołaszewska et al. (2018).

Active aeration during storage is among the gentlest methods for maintaining the required grain quality during storage. It has been found that the efficiency in air distribution is significantly affected by positioning of the air inlets in relation to the container shape. A positive effect has been shown in increasing the area of an aerated base versus increasing the air pressure through a smaller area (Katchatourian & Binelo, 2008). Aerated grain in large containers or indoor warehouses should not be considered as homogeneous material. The degree of permeability in a layer and the pressure gradient are dependent on the vertical position in the material as well as the layer height (Bradna et al., 2018).

Using mathematical modelling of heat transfer in steel silos showed that the temperature of the grain is influenced by the wall temperature, the height of grain layer and the distance between the grain and the wall. The effect of wall temperature on temperature distribution in silos is significant (Ledao et al., 2016) and, according to Foura-Belaifa et al. (2011) the seed germination is highly dependent on storage time.

It is possible to use a wide range of technologies for post-harvest grain treatment. One of them is the use of infrared radiation. When using infrared radiation to dry maize grains, a reduction in microbial load was demonstrated at a certain intensity and duration of action. Thus, the growth of moulds was prevented with a consideration to minimal energy consumption (Wilson et al., 2017). Among others, the quality of grain is also affected by insect contamination, resulting in increased temperature and moisture of the stored commodity (e.g. food wheat) (Four-Belaif et al., 2011). In contrast, Afzal et al. (2017) admitted that, when stored in big bags in subtropical areas, insects may help preserve lower grain moisture in summer and autumn months. The storage of stabilized grain in hermetically sealed plastic bags has proven to be successful because the process of breathing consumes oxygen and produces carbon dioxide, thereby fostering a suitable environment for preserving grain quality. These storage conditions depend not only on the type of grain, stored temperature and humidity, but also on the permeability of the plastic bag and its location in the storage area (Arias, et al. 2013).

MATERIALS AND METHODS

The impact of outdoor conditions on the microclimate inside a tower fabric silo was monitored on a farm in the South Moravian Region of the Czech Republic (geographic coordinates 49.041598, 16.862273). The container was a cylindrical tower made of steel wire net with a diameter of 4 m and a height of 6 m. The steel net was lined with a textile layer of woven polypropylene fabric with areal weight 200 g m^{-2} which enabled passive aeration. This type of container is used most frequently for spelt grain. In the period under review (2017), 55 tons of spelt were stored in the silo. For comparison, another sample with the same spelt variety was stored in a flexible intermediate bulk container (FIBC) bag with flat bottom, open top and equipped with 4 rigid polypropylene handles for manipulation. The physical size of the bag was 90×90×110 cm, complying with the standard ČSN EN ISO 21898. The bag was located in the attic space of a former dairy cow stall, which now serves as a storage area for bedding material and feed. This type of storage bag was made of the same properties of the textile as the silo liner for comparison. Spelt has been deliberately chosen for its good thermal insulation properties and low moisture at harvest. Stabilized grain with 12.3% moisture was used.

Figure 1. The storage of spelt grain in a FIBC bag (on the left) and in a tower silo with steel wire construction and textile liner (on the right).

Platinum temperature sensors were installed in both structures to measure the temperature inside the grain layer, in the centre of the container at a depth of 1 m below surface and connected to a datalogger (Comet S3631) for long-term measurement and recording. In both cases, the datalogger was placed within the indoor storage area to monitor the space above the stored material at a height of 2 m. A Comet S3120 datalogger was installed outside on the north side of the storage building to measure the ambient temperature and relative humidity of the outdoor environment in the immediate vicinity of the warehouse. The recording interval was set to 15 minutes. Data was continuously downloaded to a computer and processed using database tools. Statistical dependence of selected parameters was evaluated using a linear regression model.

The measured quantities were:

t_e – outside air temperature; Δt_e – daily difference of maximum and minimum of outside air temperature; t_{m1} – air temperature between the stored spelt wheat grain in the tower silo; Δt_{m1} – daily difference of air temperature maximum and minimum between the stored spelt wheat grain in the tower silo; t_{m2} – air temperature between the stored spelt wheat grain in the FIBC bag; Δt_{m2} – daily difference of air temperature maximum and minimum between the stored spelt wheat grain in the FIBC bag.

Technical data of thermo-hygrometers used: Comet R3120, S3120, S3631: temperature measuring range -30 to 70 °C, accuracy ± 0.4 °C.

Qualitative parameters of spelt samples taken from both storage areas were determined according to standardized procedures. Moisture was determined according to ISO 712:2009. Bulk density (BD) was determined according to ISO 7971-3:2009 standard using a quarter-litre measuring container. Falling number (FN) was determined according to ISO 3093:2009 using a Perten LM 3120 mill and 7 g of meal on a 15% moisture basis. Zeleny sedimentation index (SEDI) was determined according to ISO 5529:2007 using a Brabender Sedimat mill. Wet gluten content (WG) content and gluten index (GI) were determined according to the ICC Standard No. 155. Crude protein content (CPC; N×5.7) was determined by the Dumas method according to ICC Standard No. 167 using a LECO FP-528 analyser and LECO TruMac CNS. Deoxynivalenol (DON) was analysed by the ELISA method using R-Biopharm AG kits (Darmstadt, Germany), according to the manufacturer's instructions. Ochratoxin A (OTA) concentration was determined by high-performance liquid chromatography using Shimadzu Prominence HPLC equipped with Shimadzu fluorescence detector RF-10 AXL.

RESULTS AND DISCUSSION

Summaries of the average monthly outdoor temperature, average material temperature (air between grain) in the tower silo and FIBC bag and average daily temperature differences for each month are shown in Table 1.

At two-month intervals, samples of spelt from the 2016 harvest stored in the silo and in FIBC bag were taken to determine the quality parameters and contents of DON and OTA mycotoxins. The spelt had standard quality (moisture, temperature, BD, FN, CP, etc.) at the time of loading. The moisture of the samples changed during the year depending on the ambient air humidity. An increasing trend of CP content was observed during during storage, respectively an increasing trend of nitrogen content (r = 0.95, R^2 = 0.9025). Dependence on storage time and storage container was not apparent in other quality parameters. The content of mycotoxins monitored (DON, OTA) was in all cases always below the detection limit of 20 μg kg^{-1} and 0.2 μg kg^{-1}, respectively. The average values of the monitored parameters, minimum and maximum are shown in Table 2.

Table 1. Monthly average values of the observed temperatures and temperature differences during a day

Month	t_e °C	Δt_e °C	t_{m1} °C	Δt_{m1} °C	t_{m2} °C	Δt_{m2} °C
January[1)]	-4.9	4.3	3.4	0.1	-3.2	0.6
February	1.5	6.3	1.5	0.1	0.6	0.8
March	8.1	10.2	2.3	0.1	6.9	1.1
April	9.6	9.8	7.7	0.1	9.2	1.1
May	17.3	13.6	11.0	0.2	15.1	1.5
June[2)]	21.4	15.7	16.7	0.3	20.3	1.5
Total[3)]	9.4	10.2	6.5	0.1	8.4	1.1

1) January 22nd – January 31st; 2) June 6th – June 13th; 3) January 22nd – June 13th.

Table 2. Qualitative parameters of spelt stored in the FIBC bag and tower silo. Values are averages from 20 samples taken in 2017 and the minimum and maximum values achieved

Parameter	unit	FIBC bag	tower silo
Moisture content	%	12.3 (11.1–16.2)	11.5 (10.9–13.8)
DON	μg kg^{-1}	< 20	< 20
OTA	μg kg^{-1}	< 0.2	< 0.2
BD	kg hL^{-1}	76.1 (75.3–77.3)	75.6 (74.8–76.4)
FN	s	289 (281–303)	284 (275–298)
SEDI	mL	15 (14–16)	16 (15–17)
GI		20 (18–26)	21 (19–26)
WG	%	33.2 (30.4–34.4)	39.9 (37.4–41.0)
CPC	%	13.2 (12.8–13.5)	13.5 (13.2–14.0)
Germination	%	85.6 (81.0–88.0)	84.9 (81.5–87.0)

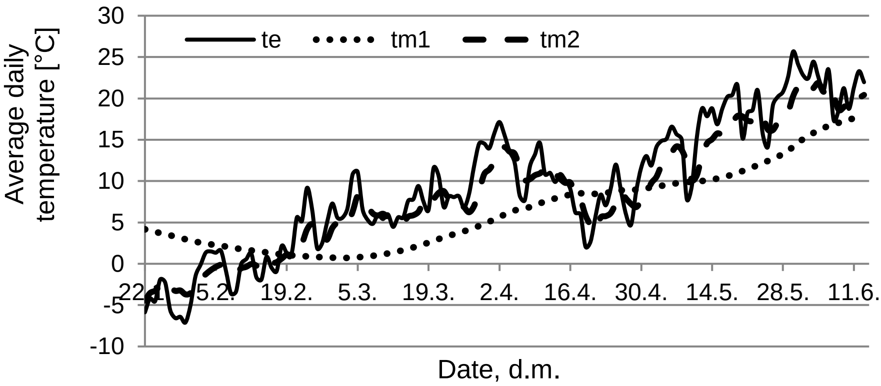


Figure 2. Comparison of the average daily outdoor temperature and average daily temperature in the spelt layer of both storage structures in 2017.

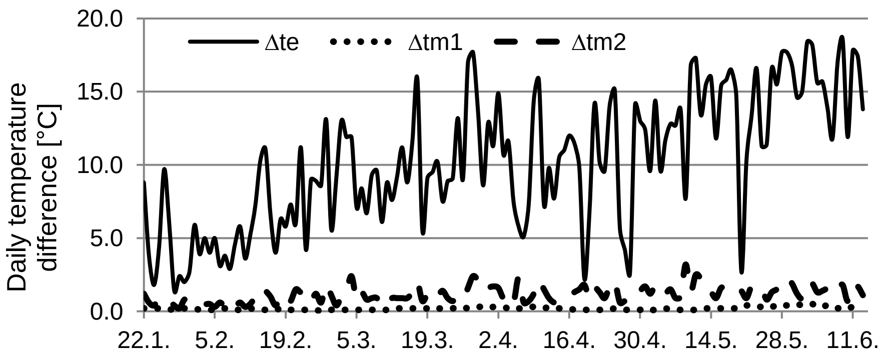


Figure 3. Comparison of the average daily outdoor temperature differences and average daily temperature differences in the spelt layer of both storage structures in 2017.

The trends in temperatures and temperature differences are shown in Table 1 and Figs 2 and 3. Generally, due to the larger amount of material and the thermal insulating characteristics of the warehouse where the silo was located, daily temperature variations in the material layer in the silo were less than 0.5 °C and the average daily temperature lags the outdoor environment. The temperature inside the material reacts less sensitively to the temperature fluctuations of the outdoors environment due to the good thermal insulation properties of the grain and warehouse.

On the other hand, in the FIBC bag the temperature inside the material responded very quickly to fluctuations in outside temperature, due to both lower volume of stored material and the location of the bag which was an attic space without any roof insulation, i.e. worse thermal insulation properties of the storage space. This experiment site was chosen deliberately to store a smaller amount of the same material than the tower silo, and to verify the effect of extreme storage conditions on the material and the rate of mould and mycotoxin development in such an environment.

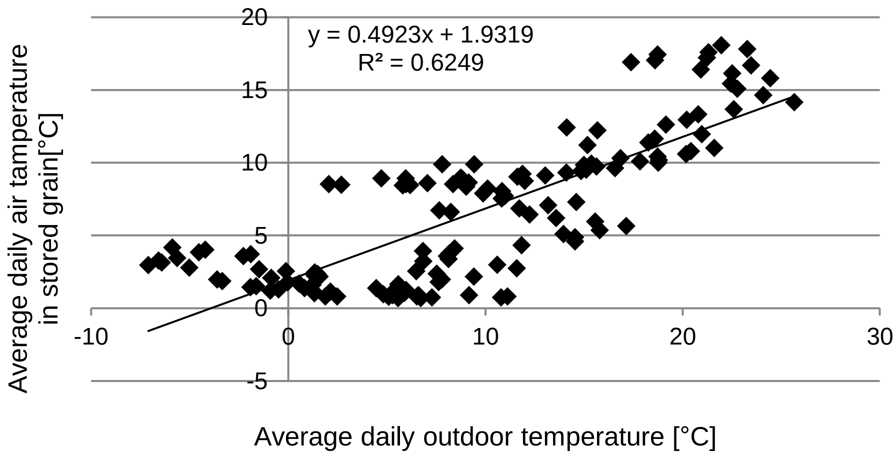


Figure 4. Comparison of the average daily outdoor temperature and average daily temperature in the spelt layer in the silo in 2017.

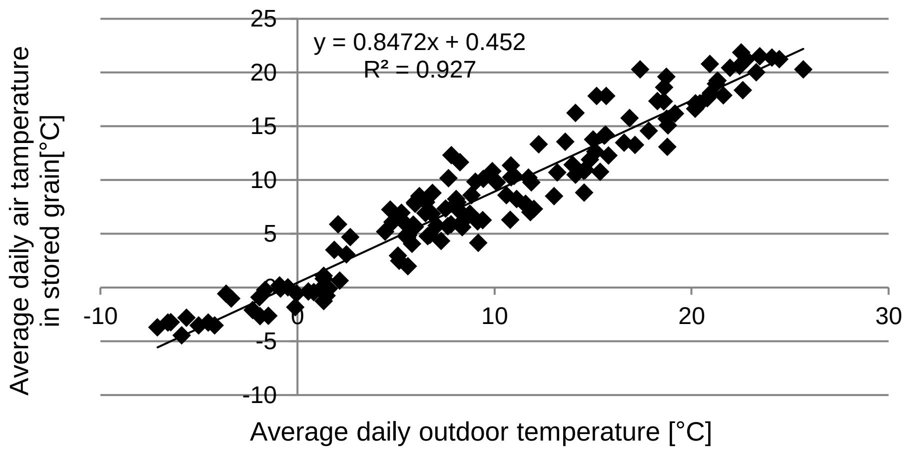


Figure 5. Comparison of the average daily outdoor temperature and average daily temperature in the spelt layer in the bag in 2017.

Dependence of the average daily temperature in the layer of the stored spelt grain on outside temperature is shown in Figs 4 and 5. Fig. 4 shows a simple linear regression model of the temperature dependence of the stored material inside the tower silo on the outside temperature. The correlation coefficient r = 0.7905 (R^2 = 0.625) shows moderate dependence in the long-term data from the observed part of the storage season. Fig. 5 shows a simple linear regression model of the temperature dependence of the stored material inside the FIBC bag on outside temperature. The correlation coefficient r = 0.9628 (R^2 = 0.927) shows almost linear correlation and a strong dependence in the long-term data from the observed part of the storage season.

CONCLUSIONS

The results show a strong dependence of the stored material temperature on the outside temperature in the case of FIBC bags (coefficient of determination R^2 = 0.927). The dependence in the case of silo storage was weaker (R^2 = 0.625). This difference is mainly due to the volume of material stored and the associated heat capacity and thermal inertia. The biological activity of the stored grains may also impact the dependence of the temperature of grains on relative humidity and grain moisture.

There was no significant change in quality parameters during the two-year storage period. Only increasing crude protein content was found. Due to the storage of healthy and cleaned spelt, the storage time in the bag and silo did not affect the content of mycotoxins, which remained below the detection limit. However, significant fluctuations in humidity and temperature of the environment can affect the mycotoxicological quality of the stored production and there is a risk of increasing the content of mycotoxins, especially when storing contaminated, uncleaned grain.

Temperature variations within the silo in the warehouse and in bags placed in an attic storage area directly under roof without any thermal insulation over six-month period have been measured and evaluated. Temperature at 1 m depth inside the layer of spelt wheat grain increased from January through June in comparison with the measured ambient temperature. This may be associated with heat of respiration of the grain together with the accumulated heat gain during daylight hours.

The use of FIBC bags is not ideal for the goal of achieving and maintaining a stable storage temperature. Especially when bags are placed in uninsulated spaces. In such cases, the microclimate inside the bag is greatly affected. Regarding their ability to maintain a stable microclimate, bags were the worse of the two storage technologies used in this study. This was due to the lower storage capacity and thus the lower capacity to accumulate and maintain heat. The solution could be simply storing bags in a warehouse with a sufficient thermal insulation, or even in combination with a microclimate control system.

ACKNOWLEDGEMENTS. This research was supported by the Ministry of Agriculture of the Czech Republic, project NAZV QJ1510204 and by an institutional project of the Ministry of Agriculture of the Czech Republic (RO0619).

REFERENCES

Afzal, I., Bakhtavar, M.A., Ishfaq, M., Sagheer, M. & Baributsa, D. 2017. Maintaining dryness during storage contributes to higher maize seed quality. *Journal of Stored Products Research* **72**, 49–53. https://doi.org/10.1016/j.jspr.2017.04.001

Arias Barreto, A., Abalone, R., Gastón, A. & Bartosik, R. 2013. Analysis of storage conditions of a wheat silo-bag for different weather conditions by computer simulation. *Biosystems Engineering* **116**(4), 497–508. https://doi.org/10.1016/j.biosystemseng.2013.10.012

Bradna, J., Šimon, J., Hájek, D. & Vejchar, D. 2018. The impact of weather conditions on microclimate in storage facilities. *Agronomy Research* **16**(4), 1580-1589. ISSN 1406-894X

Capouchová, I, Papoušková, L., Hajšlová, J., Prokinová, E., Kostelanská, M., Škeříková, A., Dvořáček, V., Pazderů, K. & Faměra, O. 2009. Risks of the occurrence of fusarium sp. for baking quality of wheat (Rizika výskytu fuzarióz pro pekařskou jakost pšenice) *Úroda* **12**, 521–526.

Czech Republic Government Decree Act No. 333/1997 Sb. of 12 December 1997. 1997. Collection of Laws of the Czech Republic. 111. 6786–6809.

Czech Republic Government Decree Act No. 129/2012 Sb. of 4 April 2012. 2012. Collection of Laws of the Czech Republic. 48.1962–2201.

ČSN EN ISO 21898 (2006). Packaging - Flexible intermediate bulk containers (FIBCs) for non-dangerous goods (Obaly - Flexibilní středně objemové vaky (FIBC) pro jiné než nebezpečné věci). Prague: Czech Standards Institute.

Fourar-Belaifa, R., Fleurat-Lessard, F. & Bouznad, Z. 2011. A systemic approach to qualitative changes in the stored-wheat ecosystem: Prediction of deterioration risks in unsafe storage conditions in relation to relative humidity level, infestation by Sitophilus oryzae (L.), and wheat variety. *Journal of Stored Products Research* **47**(1), 48–61.

Hammami, F., Ben Mabrouk, S. & Mami, A. 2017. Numerical investigation of low relative humidity aeration impact on the moisture content of stored wheat. *International Journal of Modeling, Simulation, and Scientific Computing* **8**(2), 1740002.

Khatchatourian, O.A. & Binelo, M.O. 2008. Simulation of three-dimensional airflow in grain storage bins. *Biosyst. Eng.* **101**(2), 225–238. doi: 10.1016/j.biosystemseng. 2008.06.001

Ledao, Zhang, Xi, C., Hang, L., Wei, P., Zhongjie, Z. & Guangyue, R. 2016. Experiment and simulation research of storage for small grain steel silo. *International Journal of Agricultural and Biological Engineering* **9**(3), 170–178. https://doi.org/10.3965/IJABE.V9I3.1946

Polišenská, I., Pfohl-Leszkowicz, A., Hadjeba, K., Dohnal, V., Jirsa, O., Denešová, O., Ježková, A. & Macharačková, P. 2010. Occurence of ochratoxin A and citrinin in Czech cereals and comparison of two HPLC methods for ochratoxin A detection. *Food Additives and Contaminants* **27**(11), 1545–1557.

Wilson, S.A., Okeyo, A.A., Olatunde, G.A. & Atungulu, G.G. 2017. Radiant heat treatments for corn drying and decontamination. *Journal of Food Processing and Preservation* **41**(5), e13193. https://doi.org/10.1111/jfpp.13193

Żuk-Gołaszewska, K., Majewska, K., Tyburski, J. & Gołaszewski, J. 2018. Physical and technological properties of kernels and flour made from spelt grown in an organic farming system in north-eastern Poland. *Journal of Cereal Science* **79**, 501–507. https://doi.org/10.1016/j.jcs.2018.01.002.

Agronomy Research **17**(6), 2211–2219, 2019
https://doi.org/10.15159/AR.19.207

Experimental study of the distribution of the heights of sugar beet root crowns above the soil surface

V. Bulgakov[1], M. Arak[2], A. Boris[3], M. Boris[4], V. Bandura[5] and J. Olt[2,*]

[1]National University of Life and Environmental Sciences of Ukraine, 15 Heroyiv Oborony street, UA 03041 Kyiv, Ukraine
[2]Estonian University of Life Sciences, Institute of Technology, 56 F.R. Kreutzwaldi street, EE 51006 Tartu, Estonia
[3]National Scientific Centre, Institute for Agricultural Engineering and Electrification, 11 Vokzalna street, Glevakhcha-1, Vasylkiv District, UA 08631 Kiev region, Ukraine
[4]Podolski State Agricultural and Technical University, 13 Shevchenko street, Kamenetz-Podilsk, UA 32300 Khmelnytsky region, Ukraine
[5]Vinnytsia National Agrarian University of Ukraine, 3 Soniachna street UA21008 Vinnytsia, Ukraine
[*]Correspondence: jyri.olt@emu.ee

Abstract. The results of experimental studies and operational tests of the sugar beet harvesting process carried out recently reveal that the latest models of beet harvesters produced in Europe and America cause considerable loss of the sugar-bearing mass. The source of this loss is mainly the poor topping of the crowns of standing sugar beet roots, more specifically the excessively low point at which the tops are cut off, which results in the straight out loss of sugar-bearing mass. Thus, there is need to search for such engineering solutions that would avoid both the loss of sugar-bearing mass and the presence of residual haulm on the roots. The aim of this study was to reduce the loss of sugar-bearing mass in the process of topping sugar beet root crowns. The results of this research into the distribution of the heights that root crowns protrude above the soil surface have confirmed the hypothesis that it follows the normal distribution. Based on the results, it has been established that this distribution has the following statistical parameters: mean deviation a = 20–30 mm, mathematical expectation m = 40...60 mm. The laboratory unit developed for this work and the field studies performed with it have provided sufficient evidence to develop a new system to automatically adjust the topping height on state-of-the-art root crop harvesters.

Key words: sugar beet, harvesting, loss, sugar-bearing mass, height control.

INTRODUCTION

Sugar beet growing is a strategic branch of the agricultural industry in the leading production states of Europe, America and China (Spiess & Diserens, 2001; Pidgeon et al., 2004; Gruber, 2005; Jansen & Stibbe, 2007; Wu et al., 2013). Harvesting is one of the critically important operations in the production of sugar beet, because at this stage a significant part of the crop can be lost (Khelemendik, 1996; Lammers & Schmittmann, 2013; Zang et al., 2013).

In view of the fact that root crop harvesting comprises the operations of topping the roots, lifting them and cleaning, the loss of harvested roots is determined by quite a number of factors (Smith et al., 1999; Bentini et al., 2005; Bulgakov et al., 2017). Emphasis should be placed on the most significant of these, which is the loss due to the unsatisfactory performance of the beet harvesters at the stages of topping the standing roots and the subsequent lifting of the roots out of the soil (Adamchuk et al., 2013; Zang et al., 2013; Bulgakov et al., 2016). An increase in the contamination by haulm waste in the sugar beet roots by just 1% over the accepted standard rate reduces the sugar recovery by 0.1%, while in the storage of roots in clamps prior to their processing, a haulm content of about 4% causes the daily loss of sugar to reach 0.02% (Bulgakov, 2013; 2016). In addition to that, sugar beet harvesters produced in Europe and America generate substantial loss of sugar-bearing mass from the excessively low shearing of root crowns (when a considerable part of the root crown which contain a large amount of sugar is cut off with the haulm).

Therefore, one of the current research options is to find ways to reduce the mentioned losses. There are many types of equipment designed to detect the root crown surface, but beet harvesters that perform topping without detectors have become the most common currently used equipment (Bulgakov et al., 2013). The current state-of-the-art sugar beet harvesters mostly employ rotor-type topping units, which cut off the tops of the roots at the same height relative to the soil surface. The selection of the height to cut off the tops is made following the generally accepted recommendations, but in practice they are often difficult to follow, in view of the probabilistic nature of the distribution of the height of top of the beets above the ground level, from which the root crowns protrude (Zhang et al., 2013). Despite that, users of the sugar beet harvesting machinery are always faced with the problem of selecting the appropriate height at which to shear the root crown under specific operational conditions. The different ways the root crowns protrude is important because that is what actually defines the crop yield index. In most cases, the problem is dealt with using rule-of-thumb methods (including visual assessment of the performance of the haulm gatherer following several trial runs). These might have to be repeated several times which is wasteful expenditure of work time, and consequently_reduces harvesting productivity, but does not guarantee a high level of accuracy in regard to harvesting the whole beet growing estate. Also, with this approach, it is possible to select the incorrect shear height, which will result in considerable loss of sugar-bearing mass. Therefore, it seems expedient to develop an automated controller, which would provide a solution to the problem of selecting shear height, setting the height automatically in the process of operating the haulm gatherer. To engineer the said controller, it is first necessary to find out the type of statistical distribution that describes the height that beet root crowns protrude above the soil surface. This issue has been tackled for many years by a number of researchers (Adamchuk et al., 2013; Bulgakov et al., 2018; Bulgakov et al., 2019) and they have predominantly favoured the normal (Gaussian) distribution. But the dependability of their results has been questioned because of the impossibility of obtaining sufficiently large samples by sampling and manual measurement.

MATERIALS AND METHODS

For an acceptably dependable assessment of the distribution of the height of sugar beet crowns in a field (distribution of plants in a row, distribution of height of root crowns above the soil, and loss during the harvesting) authors developed a laboratory-field unit (Fig. 1). The unit has been used in this research with the aim of reducing the loss of sugar-bearing mass incurred in the process of topping sugar beet roots. It involved the development of the technology for automated correction of the topping height.

The results of the accomplished measurements were processed with the use of recognised statistical methods on a PC. The main point of the statistical processing consisted of finding out the type of distribution that the measured heights to which the root crowns protruded above the soil surface and its statistical characteristics m and σ. After that, a forecast of the loss of sugar-bearing mass and the amount of residual haulm on the roots in relation to the height of topping without detecting could be prepared using the established model (Bulgakov et al., 2013). The input parameters of the model were related to characteristics of the root, the plantation conditions during the harvesting operations, and process control. The main parameters of the root included d_1, h_i (Fig. 2). The other parameters were derivative and could be calculated with the use of the relations provided in the papers by Bulgakov et al. (2013; (2019). The main field parameters included: m and σ – respectively, the statistical expectation and the standard deviation of the root crowns protrude above the soil surface (in case its distribution followed the normal probability); Q – was yield of roots per unit area (hectare); and N – number of roots in one hectare. The process parameters included height of topping without sensing h_z. The output parameters in the model were the direct values of the work process: lost sugar-bearing mass B and amount of residual haulm on the roots G, or their representation in terms of percentage of the total mass of roots. For modelling the process of defoliating the root crown, the geometrical model devised in the paper (Bulgakov et al., 2013) was used. The essence of the model consisted of determining the volume and mass of the crown of a single root with the use of the geometrical relations set out in Figs. 2, 3, 4. If the distribution of the heights by which root crowns protruded was known, the lost mass and residual haulm in relation to the cutting height could be forecast (Bulgakov, 2018). In order to change from a single root to the whole sample of roots in accordance with the above-mentioned technique, integration was carried out over the whole range of root crown heights taking into account the probability of occurrence of each value of the root crown height. The algorithm for integrating the losses in sugar-bearing mass and the amounts of residual haulm on the roots in relation to their position with respect to the soil surface and the topping plane was implemented in the computer program composed with the MatLab software.

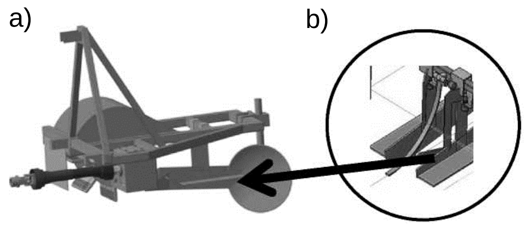


Figure 1. Laboratory unit (3D model) for measurement of height that sugar beet roots protrude above the soil level (a) and layout of its measuring unit (b).

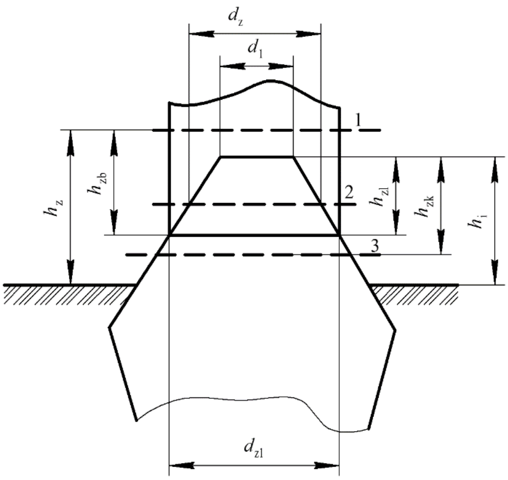


Figure 2. Geometrical model of sugar beet root crown that has bottom line of the haulm above soil surface level: h_i – root protrusion height (mm); h_{zl} – distance from top of root crown to bottom line of haulm (mm); h_z – height of topping without sensing (mm); h_{zk} – distance from top of root crown to topping plane (mm); h_{zb} – distance from topping plane to haulm bottom line (mm); G – haulm residual on roots (kg); B – loss of sugar-bearing mass (kg); ρ_b and ρ_b – densities of haulm and root (kg m^{-3}); d_1 – root crown top diameter (mm); d_z – root crown diameter in topping plane (mm); d_{zl} – root crown diameter in haulm bottom plane (mm):

Topping plane 1 is situated above the top of the root crown: In this case there is no loss of sugar-bearing material but haulm left on the root:

$$h_{zk} = 0, \tag{1}$$

$$h_{zb} = h_{zl} - h_{zk} + h_z, \tag{2}$$

$$B = 0, \tag{3}$$

$$G = \frac{\pi \cdot h_{zk} \cdot \rho_b \cdot d_{z1}^2}{4} - \frac{\pi \cdot \rho_b \cdot h_{ZL}\left(d_1^2 + d_1 \cdot d_{z1} + d_{z1}^2\right)}{12}. \tag{4}$$

Topping plane 2 is situated below the root crown top:

$$h_{zk} = h_i - h_z, \tag{5}$$

$$h_{zb} = h_{zl} - h_i + h_z, \tag{6}$$

$$B = \frac{\pi \cdot h_{zk} \cdot \rho_k \left(d_1^2 + d_1 \cdot d_{z1} + d_{z1}^2\right)}{12}, \tag{7}$$

$$G = \frac{\pi \cdot h_{zb} \cdot \rho_b \cdot d_{z1}^2}{4} - \frac{\pi\left(h_{zl} - h_{zk}\right)\left(d_1^2 + d_1 \cdot d_{z1} + d_{z1}^2\right)}{12}. \tag{8}$$

Topping plane 3 is situated below the haulm bottom plane:

$$h_{zk} = h_i - h_z, \tag{9}$$

$$h_{zb} = 0, \tag{10}$$

$$B = \frac{\pi \cdot h_{zk} \cdot \rho_k \left(d_{z1}^2 + d_z \cdot d_{z1} + d_z^2\right)}{12}, \tag{11}$$

$$G = 0. \tag{12}$$

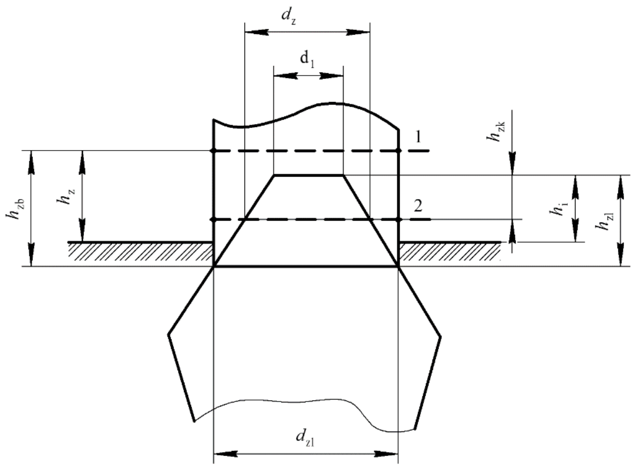


Figure 3. Geometrical model of sugar beet root crown that has bottom line of haulm below soil surface level:

Topping plane 1

$$h_{zk} = 0, \tag{13}$$

$$h_{zb} = h_{zl} - h_{zk} + h_z, \tag{14}$$

$$B = 0, \tag{15}$$

$$G = \frac{\pi \cdot h_{zb} \cdot \rho_b \cdot d_{zl}^2}{4} - \frac{\pi \cdot \rho_b \cdot h_{ZL}\left(d_1^2 + d_1 \cdot d_{zl} + d_{zl}^2\right)}{12} \tag{16}$$

Topping plane 2

$$h_{zk} = h_i - h_z, \tag{17}$$

$$h_{zb} = h_{zl} - h_i + h_z, \tag{18}$$

$$B = \frac{\pi \cdot h_{zk} \cdot \rho_k \left(d_1^2 + d_1 \cdot d_{z1} + d_{z1}^2\right)}{12} \tag{19}$$

$$G = \frac{\pi \cdot h_{zb} \cdot \rho_b \cdot d_{z1}^2}{4} - \frac{\pi\left(h_{ZL} - h_{zk}\right) \cdot \left(d_1^2 + d_1 \cdot d_{z1} + d_{z1}^2\right)}{12} \tag{20}$$

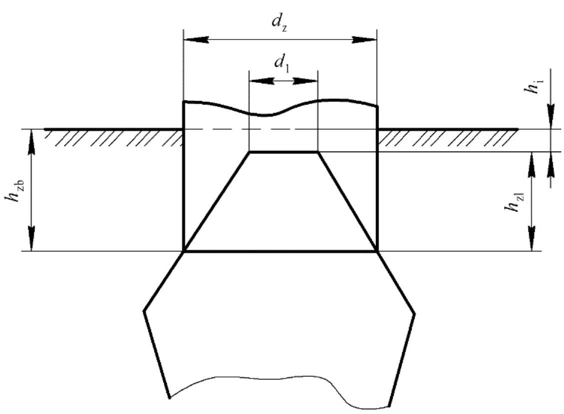


Figure 4. Geometrical model of sugar beet root crown that has top of root crown below soil surface level:

Topping plane 1

$$h_{zk} = 0, \tag{21}$$

$$h_{zb} = h_{zl} - h_{zk} + h_z, \tag{22}$$

$$B = 0, \tag{23}$$

$$G = \frac{\pi \cdot h_{zb} \cdot \rho_b \cdot d_{z1}^2}{4} - \frac{\pi \cdot \rho_b \cdot h_{ZL} \left(d_1^2 + d_1 \cdot d_{z1} + d_{z1}^2 \right)}{12}. \tag{24}$$

As the first step, the authors developed the functional structure of such an experimental unit including data input and output modules, measuring unit (Fig. 5) control module, transducer module for logging the planting parameters (the positioning of sugar beet roots in the seeding row). An algorithm was also devised to control the process of measuring the height of the root crowns above the soil level.

The structural layout of the actual laboratory unit for the performance of field experiments includes a system of gyroscopes and accelerometers engineered to investigate the impact of the beet harvester's oscillations on the stability of performance of the sugar beet root harvesting implements.

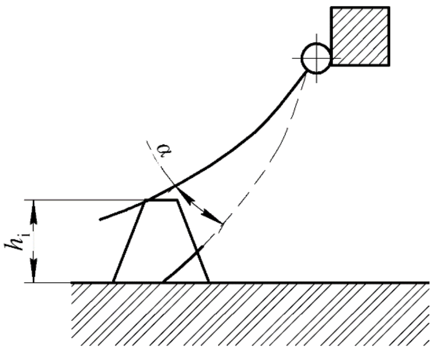


Figure 5. Schematic model of measuring root crown protrusion.

Figure 6. Experimental unit during field study of root crown protrusion above soil surface level.

The authors also developed the structural layout of the measuring unit and manufactured the electronic module to control the process of measuring the position of the sugar beet root crown above the level of soil surface. The measuring unit included sugar beet root detection feelers and other feelers that identify the position of the machine's wheelbase and the height that the crown of the sugar beet root protrudes above the soil surface. The height that the root crowns protrude above the soil surface was measured by the feeler (Fig. 5) that would deflect through an angle α, when it interacted with a root protruding to a height of hi. The deflection angle of the feeler was registered by the encoder comprising a magnetic disc and two A3144 Hall-effect sensors. At the moment when the magnet passed through the sensor zone, the sensors generated digital impulses, the number of which was proportional to the angle of deflection achieved by the feeler. The signal from the encoder was transmitted to the E-14-440 digital input-output board of the LCard brand. The E-14-440 module was connected through a USB connection to the PC with the LGraf program installed. In the process of measurement, the program generated a file with the data on the heights that the root crowns protruded above the soil surface. After collecting a sufficient number of measurements (over 50,000 roots), the file was exported into the Octave 4.0 problem-solving environment and there the loss in sugar-bearing mass and the amount of residual haulm on the roots were determined. The field studies were carried out with a single-row unit attached to a propulsion and power module (Fig. 6). The research was conducted in different beet fields with different biological yields of sugar beet roots and haulm, different geometry of the field surface, and a range of mechanical and physical properties of the soil. At the same time, graphs were plotted based on the results of processing a large sample (50 thousand measurements) on a PC with the use of dedicated MatLab software.

RESULTS AND DISCUSSION

The results of the research into the distribution of height of beet root crowns protruding above the soil surface are presented in Fig. 7. These graphs display a high degree of dependability and show the distribution of the heights that sugar beet root crowns protrude above the soil surface, depending on the intervals h indicated on the x-axis. Using a mathematical model (Adamchuk et al., 2013), the relationship between the loss of sugar-bearing mass and the height of topping sugar beet root crowns without sensing was obtained. Fig. 8 represents an example of the relationship (for one specific case) under the following distribution parameters: $m = 40$ mm and $a = 20$ mm. Nevertheless, in the same field these parameters were shown to vary within the limits indicated in Fig. 7. Therefore, the statistical parameters of the distribution should be monitored by a dynamic process. With their variation the graph of sugar-bearing mass (Fig. 8) as well as the optimal value h_z change correspondingly.

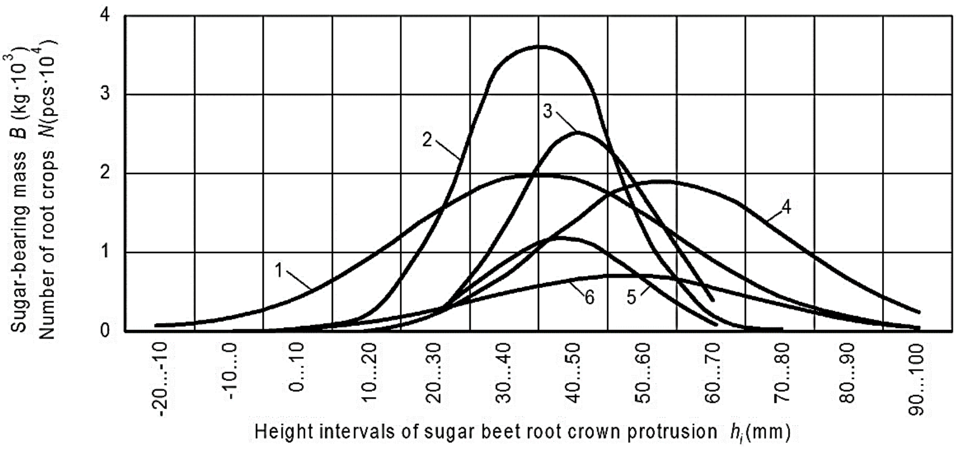


Figure 7. Distribution of heights of sugar beet root crown protrusion above soil surface where $m = 40$ mm: N – number of roots: 1) $\sigma = 10$ mm; 2) $\sigma = 20$ mm; B_z – sugar-bearing mass above ground: 3) $\sigma = 10$ mm; 4) $\sigma = 20$ mm; B_{3n} – sugar-bearing mass above ground containing haulm: 5) $\sigma = 10$ mm; 6) $\sigma = 20$ mm.

Fig. 8 can forecast the loss of sugar beet root mass and the rate of contamination of roots with haulm under specific conditions, once the statistical distribution parameters (*m, a)* have been established using the mathematical model presented by Adamchuk et al. (2013). That, in its turn, will allow an automated system of evaluating the parameters of sugar beet roots and promptly adjusting the height of shearing the haulm off the sugar beet rootsto be developed with the aim of reducing the loss of sugar-bearing mass.

Lines 1–2 in Fig. 7 show the distribution of the protrusion heights on the intervals, lines 3–4 the distribution of sugar-bearing mass on the protrusion height intervals, lines 5–6 the distribution of the sugar-bearing mass that needs cleaning from haulm.

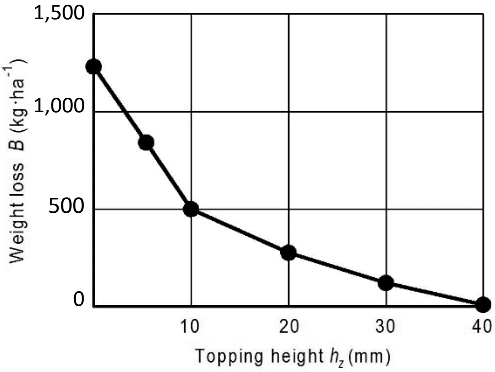


Figure 8. Relationship between loss in sugar-bearing mass (kg) and height of topping root crowns (mm).

CONCLUSIONS

1. The results of this research into the distribution of the heights that sugar beet root crowns protrude above the soil surface confirm the hypothesis that it follows the normal distribution.

2. The laboratory unit that was developed and the experimental study carried out with it provides the basis for the design of a system to automatically control the height of shearing haulm from the top of the roots in state-of-the-art root crop harvesters.

REFERENCES

Adamchuk, V., Boris, A., Bulgakov, V. & Boris, M. 2013. The theoretical study of the cleaner of sugar beet roots heads. Zemes ukio inzeneriia, mokslo darbai / *Agricultural Engineering*, *Research Papers* **45**(3), 13–19.

Bentini, M., Caprara, C. & Rondelli, V. 2005. Mechanical properties of sugar beet roots. *Transactions of the American Society of American Engineers* **48**(4), 1429–1439.

Bulgakov, V., Adamchuk, V., Arak, M. & Olt, J. 2018. The theory of cleaning the crowns of standing beet roots with the use of elastic blades. *Agronomy Research* **16**(5), 1931–1949. doi: 10.15159/AR.18.213

Bulgakov, V., Adamchuk, V., Arak, M. & Olt, J. 2017. A theoretical study of haulm loss resulting from rotor topper oscillation. *Chemical Engineering Transactions* **58**, 223−228. doi: 10.3303/CET1758038

Bulgakov, V., Boris, M. & Boris, A. 2013. Theoretical investigations of leaf stripper heads of roots. Zemes ukio inzeneriia, mokslo darbai / *Agricultural Engineering*, *Research Papers*, **45**(2), 46–53.

Bulgakov V., Ivanovs S., Golovach I. & Ruzhylo Z. 2016. Theoretical investigations in cleaning sugar beet heads from remnants of leaves by cleaning blade. **In**: *Proceedings from 15th International Scientific Conference Engineering for Rural Development*, Jelgava, May 25–27, Jelgava: 1090–1097.

Bulgakov, V., Pascuzzi, S., M. Arak, M., Santoro, F., Anifantis, A.S., Ihnatiev, Y. & Olt, J. 2019. An experimental investigation of performance levels in a new root crown cleaner. *Agronomy Research* **17**(2), 358–370. doi: 10.15159/AR.19.132

Gruber, W. 2005. Trends in sugar beet harvesting. *Landtechnik* **60**(6), 320–321.

Jansen, R. & Stibbe, G. 2007. Impact of plant breeding on the profitability of sugar beet production. *International Sugar Journal* **109**(1300), 227–233.

Khelemendik, N. 1996. Increasing the Mechanical and Technological Efficiency of the Labour Consuming Processes in Beat Growing. A thesis for applying for the degree of doctor philosophy in agricultural engineering, Ternopol Instrumental Engineering Institute, 48 pp. (in Ukrainian).

Lammers, P.S. & Schmittmann, O. 2013. Testing of sugar beet harvesters in Germany 2012. *International Sugar Journal* **115**(1370), 100–106.

Pidgeon, J.D., Jaggard, K.W., Lister, D.H., Richter, G.M. & Jones, P.D. 2004. Climatic impact on the productivity of sugarbeet in Europe. *Zuckerindustrie* **129**(1), 20–25.

Smith, J.A., Yonts, C.D. & Palm, K.L. 1999. Field loss from sugar beet harvest operations. *Applied Engineering in Agriculture* **15**(6), 627–631.

Spiess, E. & Diserens, E. 2001. Zuckerruben: Erntetechnik und Bodenschutz. Vielseitige Wechselwirkungen zwischen Technik, Erntequalitat und Okologie. Eidgenssische Forschungsanstalt fur Agrarwirtschaft und Landtechnik (FAT), FAT-Berichte Nr. **567**, Tanikon TG: Ettenhausen.

Zang, G., Xu, W. & Fan, S. 2013. Analysis and parameter optimization of adjustable beet top cutting mechanism. Nongye Gongcheng Xuebao/*Transactions of the Chinese Society of Agricultural Engineering* **29**(18), 26–33. doi: 10.3969/j.issn.1002–6819.2013.18.004

Wu, H., Hu, Z., Peng, B., Wang, H. & Wang, B. 2013. Development of auto-follow row system employed in pull-type beet combine harvester. Nongye Gongcheng Xuebao/*Transactions of the Chinese Society of Agricultural Engineerin*g **29**(12), 17–24.

Agronomy Research **17**(6), 2220–2232, 2019
https://doi.org/10.15159/AR.19.214

Vegetative growth response of beets and lettuce to stored human urine

J.A. Chávez[1], J.L. Alcántara-Flores[2,*], R.C. Almiray-Pinzón[3], E. Díaz-Cabrera[3], R. Pérez-Avilés[2], M.E. Patiño-Iglesias[2] and M.A. Mora-Ramírez[3,*]

[1]University of Puebla, Faculty of Phys. Sci. Math., Avenida San Claudio y 18 Sur, Col. San Manuel, Edif., FM1-101B, Ciudad Universitaria, postal code 72570, Pue., Puebla, México
[2]University of Puebla, Science Institute, Av. San Claudio, Edif FM8 111-D, San Manuel, 72570 Puebla, Pue., México
[3]University of Puebla, Faculty of Chemistry, Department of Physico Mathematics, Av. San Claudio y 18 Sur, 72570 Puebla, Pue.,
[*]Correspondence: jlaf7@yahoo.com.mx; marco.x.mora@gmail.com

Abstract. In this work, we present the experimental results of the effect of stored human urine (SHU) on the growth of beets (*Beta vulgaris* L) and lettuce (*Lactuca sativa* L). We apply different amounts of SHU according to the recommended dose of nitrogen, considering soil from farmland and vermiculite as substrates. The last allows us to determine with high precision the isolated effect of SHU over the vegetative development of beet plants, without considering other nutrients present in common soils. Experimental results demonstrate that the application of SHU has no significant effects on lettuce vegetative growth under our soil conditions. In contrast, SHU can be used successfully as a fertilizer of beets. The optimum dose was found at 120 kg N ha^{-1} and resulted in average dry weight of 125 g. However, if the dose exceeds the optimum levels, the growth of the plant is inhibited. Beets fertilized with SHU does not pose any hygienic risk for human consumption. Our findings represent a promising alternative to propose expanding the use of SHU as fertilizer in medium-sized greenhouses and to provide benefits to families in rural areas, with little or no available water supplies.

Key words: lettuce, beets, human urine, fertilizer, nitrogen.

INTRODUCTION

The production of food in the field requires water and fertilizers. However, there are large areas of water scarcity in the world that affect millions of people, many of whom are poor and disadvantaged. Therefore, there is a growing need to use fertilizer and water in smarter ways to improve the yield production of vegetables. Among other requirements, the macronutrients as nitrogen (N), phosphorus (P), and potassium (K) are essential for the plant development, including its growth and nutritional content (White et al., 2010; Vatansever et al., 2017). Therefore, fertilizers become indispensable for agriculture practices. Except for legumes (Bibi et al., 2016), many crops have benefited from fertilization with nitrogen (Pavlou et al., 2007; Liu et al., 2014), but in parallel several environmental and health problems are attributed to the misuse and excessive use of commercial fertilizers (van der Ploeg et al., 2001). The search for alternative fertilizers, economically accessible for people living in rural regions, suggests the application of human urine as a nitrogen source. In the literature there is a vast amount of work dealing with the treatment and utilization of human urine for agricultural purposes (Maurer et al., 2006; Pradhan et al., 2007; Pradhan et al., 2010; Richert et al., 2010; Wohlsager et al., 2010; Makaya et al., 2014; Andersson et al., 2015). The urine is a natural by-product of human metabolism, constituted by 91–96% of water, salts, organic compounds. Urine is widely available; an adult person produces around 1.3 L of urine per day. Likewise, the chemical composition of human urine depends on diet and health factors. Urine contains large quantities of N (90%), K (65%), and P (55%) (Heinonen-Tanski et al., 2005), which are favorable for vegetative plant development (Nagy & Zseni, 2017). Perhaps, one of the main concerns of consuming vegetables fertilized with human urine are the hygienic and taste properties. For the first issue, the urine of healthy persons is considered sterile up to it flows through the urethra. It is well known that urethra is covered by epithelial cells that contain bacteria (Colleen et al., 1980). Usually, freshly dejected urine contains < 10,000 bacteria in one mL (Tortora et al., 1989). The persistence of bacteria in urine depends on pH (Thornton et al., 2018), while the viruses are more frequently related to temperature (Höglund et al., 2001; Vinnerås et al., 2008). The pH of freshly dejected urine is ~6.5, and after urea hydrolysis, urine could reach up a pH ~9 enabling urine sanitization (Höglund, 2001; Senecal et al., 2018; Thornton et al., 2018). Therefore, it is recommended to store urine for up to 6 months to guarantees an increase in pH, and consequently, neutralize all pathogens (Bischel et al., 2015). In this regard, human treated urine can be seen as a low-cost solution, available on each house, and requiring a minimum amount of water.

The goals of this research are to evaluate the use of stored human urine as fertilizer on (i) vegetative development of beet (*Beta vulgaris* L.) and lettuce (*Lactuca sativa* L.) plants. (ii) Test the hygienic quality of the plants to ensure no risk for human consumption and (iii) determine to some extent if the farm soil is suitable for the agriculture production of these vegetables. This paper focuses on testing the effect of different doses of nitrogen contained in urine on the growth of plants. We compare the yields of beets when vermiculite and farm soil substrates are used.

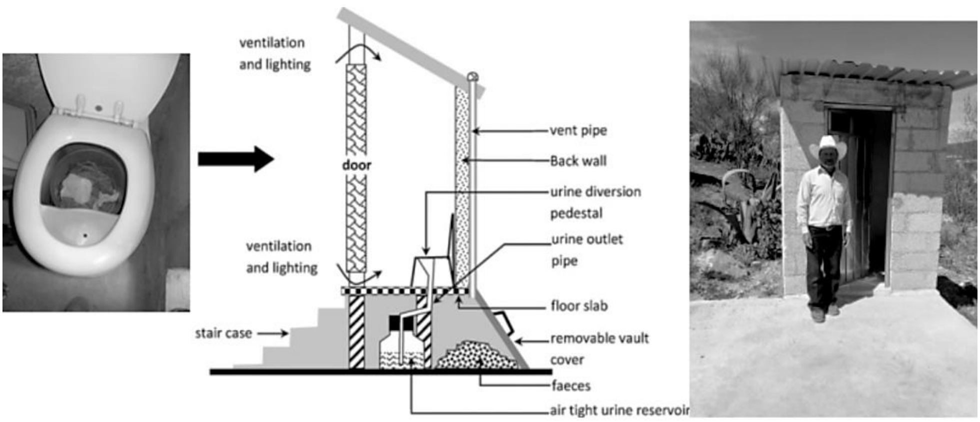
The state of Puebla occupies the second and third place in beet and lettuce production at national level. These plants grown in domestic or regular size farms, with the potential of benefiting families in rural areas of Puebla. The quality of the irrigation water compromises the production of these vegetables. In Puebla, only one-third of the 2,248 municipalities carry out water treatment. Then it is crucial to ensure and measure the hygienic quality of urine, substrate, harvested plants, and water used for irrigation.

MATERIALS AND METHODS

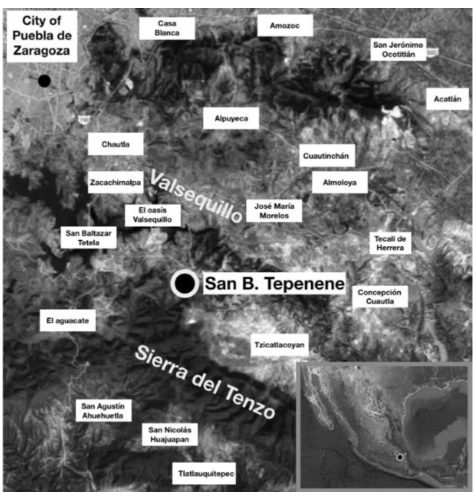
Temporal and spatial domains for the study

We conducted experiments on vegetative development in three stages. From July to August 2017 (60 days) the lettuce growth (weight, size) was studied using farm soil as a substrate. Two additional experiments were performed to evaluate the growth (weight) of beets. From September to December 2017 (73 days) using farm soil as substrate, and from June to August 2018 (62 days) using vermiculite as a substrate. The experiments were carried out at the Greenhouse of the University of Puebla, located at 19.00 °N latitude and -98.20ºW longitude with an area of 24 m^2 at an altitude of 2,135 MASL. These facilities are suitable for the development of plants, equipped with the essential requirements to grow plants, including an entrance locker to minimize contamination by external agents.

The urine and soil samples were collected from San Bernardino Tepene, located in the region between the Sierra del Tentzo and the Valsequillo depression, as shown in Fig. 1. The area, in general, presents rugged hills, calcareous and barren, limestone, and arid hills that rise on the Poblana plateau. According to the World Reference Base (Martínez-Villegas, 2007; FAO, 2015), these semi-arid areas are classified as regosols, which usually allow for incipient production and are complicated to handle. On average, these soils have between 167 to 200 mg N m^{-3} (Batjes, 1996; Premanandarajah, 2017). Besides, these soils have a low moisture retention capacity. Consequently, techniques such as drip or spray are required, which is not an economically viable alternative for the inhabitants. The weather in this region is identified as CWA (Köpen et al., 1918); this corresponds to temperate sub-humid climate, with rains (800 mm yr^{-1}) between May and September. The mean temperature of the warmest month (May) exceeds 23 °C.



a)



b)

Figure 1. a) From left to right: UDDT, schematic design and, the facility installed in b) San Bernardino Tepenene, Puebla (18.87°N, -98.09ºW), ubicated among the Sierra del Tenzo and Valsequillo depression.

Sampling

The farm soil and commercial vermiculite were the two types of substrates used in this project. As it is intended that the results of this work can be applied in situ, soil samples were collected directly from the family farm of San Bernardino Tepenene. Soil samples were taken later to our greenhouse at the University of Puebla. The other substrate used in our experiments was the Vermiculite, collected from the Agronomy Department of the University of Puebla, in the commercial form 'Agrolite' in 100 L bags, and later analyzed and put in the corresponding 6 L pots.

Lettuce and beets seedlings were carefully obtained two weeks after germination. Intending to simulate the farmer's behavior of acquiring seedlings for cultivation, and at the same time, it is more favorable to start the experiment with ready-made seedlings than trying first to achieve germination of the corresponding seed in the pots. These facts are an advantage for us in comparison with the procedures of other authors (Taylor, 1997). Another essential element in our research was the water, which comes from a University cistern. Microbiological analysis and physical-chemical parameters were measured to inspect the microorganisms or variables that have relevance in the results of the experiment.

The urine was collected in a familiar ecological toilet with urine separation, known as Urine-diverting dry toilet (UDDT), and ubicated at the community of San Bernardino Tepenene in Puebla (18.87ºN, -98.09ºW). Fig. 1, a shows the UDDT, the design and the facility. These follow the literature recommendations (Larsen et al., 1997). We collected urine from all family members. Recent studies indicate that there is no difference between the effect that urine of men and women can cause in vegetable development (Duniya, 2018). Urine management and storage is based on other experiments (von Münch, 2011).

Experimental design and treatments

Sixty kilograms of the farm soil sample was deposited on a clean surface to homogenize while water was added up to reach 60% moisture in the soil sample. Each tool used for the substrate manipulation was previously cleaned to reduce the contamination vector for the plants, and avoid interference with the further microbiological analyses. The soil then was ready to be transferred into equal plastic containers, 6 L pots. These will serve, as explained later, to perform experiments with lettuce and beets. Also, we collect vermiculite in commercial form 'Agrolite' in 100 L bags. Both farm soil samples and vermiculite were subjected to various tests to measure different parameters.

It is recommended to store urine up to six months to reduce the levels of risk agents (e.g., pathogens) that could make the consumption of vegetables fertilized with urine dangerous (Wielemaker et al., 2018). Also, the World Health Organization (WHO) recommends reaching elevated pH (~9) and high ammonium ($NH4^+$) concentration, in combination with warm temperatures. For our experiment, the collected urine was stored in a sealed (20 L) container for six months at room temperature (20.6 ± 2.6 °C). The container was collocated in the shade to ensure a fresh environment to reduce the risk of nitrogen evaporation and bad odors. Hereafter, we use the term urine dose, as the amount of nitrogen per hectare (kg N ha^{-1}) supplied to the plant. The urine dose is considered our study factor; this means we analyzed the vegetative response to different doses of SHU. Based on the literature (Mnkeni et al., 2008; Andersson et al., 2015; Mamani-Mamani et al., 2015), we selected doses for our experimental vegetables. Table 1 shows the different doses applied since the first day up to the harvesting day, known as treatments, and their corresponding equivalences in liters and nitrogen grams. Table 1, a reveals four treatments (LD_1, …, LD_4) for lettuce cultivated in pots filled with farm soil, and Table 1, b presents the treatments (BD_1, ..., BD_5) for beets grown in both farm soil and vermiculite. The values summarized in Table 1, correspond to the suggested amounts of nitrogen by a hectare (kg N ha^{-1}). The equivalent doses for our plants were calculated based on our pot dimensions. Each pot has a soil surface ~0.062 m^2, in agreement withthis, we calculate the total equivalent liters (L) and grams of nitrogen (g N) that must receive each plant. The second and third columns of Tables 1, a and 1, b show the equivalences. Equivalences are intending to make more reliable the possibility of practical consulting from the population. Then each total dose is divided into three identical proportions that are supplied to plants every ~15 days (on days 1, 15 and, 30). These, in turn, were previously diluted with water (1:4), with the aim not damage the plant with the direct application of urine. Finally, pour the mixture around the base of the seedling stem.

Table 1. Treatments for cultivated (a) lettuce in farm soil and (b) beets in farm soil or vermiculite as substrate. The dose and their corresponding equivalence in each pot (~0.062 m^2) are given

a)

	Lettuce		
	(kg N ha^{-1})	(L)	(g N)
LD_1	0	0	0
LD_2	66	0.09	0.40
LD_3	132	0.18	0.81
LD_4	198	0.28	1.21

b)

	Beets		
	(kg N ha^{-1})	(L)	(g N)
BD_1	0	0	0
BD_2	30	0.04	0.18
BD_3	60	0.08	0.36
BD_4	120	0.17	0.73
BD_5	240	0.33	1.46

Experimental Units

For the first experiment, we select from a sample of 100 units the 32 of best lettuce specimens, those that present at our discretion the better vegetative development. Using the R package ′agricolae′, randomly formed four groups (LD1, LD2, LD3, LD4) corresponding to the respective doses shown in Table 1, a. These, in turn, each one consisting of (8) lettuce plants, planted in their corresponding pots filled with farm soil and positioned randomly in four rows in the table of greenhouse facility. Lettuce plants received their respective doses of SHU on days 1, 15, and 30. Likewise, each pot was irrigated with 500 mL of water, in which case the precaution was taken to return the leachate to the pot. The lettuce plants were harvested at day 60, and the dry weight of the plant, the width, and length of the leaves were measured.

Similarly, for the second experiment, we selected thirty (30) of the best beet specimens from 100 units and, form five groups, each one of 6 elements. The corresponding dosages of SHU, shown in Table 1, b, were supplied on days 1, 15, and 30. Again, each pot filled with farm soil was irrigated with 500 mL of water provided every two days. Finally, on day 73, beets were harvested, and the dry weight parameter was measured.

For the third experiment, we selected the best forty (40) beets, from a sample of 100 units, and five groups were formed, with eight members each. However, this time, beets were planted on vermiculite, and 1 mL of nutritional solution (Qfuska Foliar; 5N-15P-5K) was added on the first day. Then we add the respective 500 mL of water every two days, as well as the application of SHU on days 1, 15, and 32. Finally, harvests on day 62 and measure the dry weight of the plant.

Response variables

The response variables chosen in this work, correspond to those that best reflect and characterize the object of study. These are the dry weight of the plant and, length and width of the plant's leaf. All of them measured at the harvest day. For each leaf, dimensions were calculated using a meter rule (± 0.5 mm), and for each plant and dose, the average length and width were calculated. After measuring the fresh plant dimensions, we proceed to wash the plant and start the drying process. The process consists of collocating the plant in a paper bag inside an oven set to low heat (140 °F or 60 °C). The plants dry up them get cool and measured on a scale (Ohaus EX223 milligram laboratory balance). The same process is repeated for each plant per treatment to obtain dry weight averages. However, we must warn that there is a possibility that a few plants die during the experiment. We will make sure that this has nothing to do with the increase in the dose of urine, but other random factors. Research on the correlation of urine dose and plant development was done using statistical analysis. We explore the data distribution and performed variance analysis (ANOVA). Also, the Tukey test incorporated in analysis tools in R allows for multiple comparisons between the averages of each treatment. It was interesting to propose vermiculite as a substrate to study, without the effect of other organic substances, the correlation between the response variables, and the different doses of stored human urine. The significance level requires to have p-values > 0.05 to declare significance.

Response variables

Table 2 summarizes the measured parameters in sample elements: urine, farm soil, plants, water, and soil. The study of hygienic quality of the plants results in negative for coliforms, Salmonella, molds, and yeasts. The urine quality results, show as expected, absence of E. coli. This bacterium typically has short survival in the urine. Then, this is not a suitable indicator of fecal contamination. Other indicators in urine, like gram-negative bacteria; Salmonella, and Aerobic mesophilic bacteria (BMA) resulted in negative, under the standard norms, indicating a low risk of gastrointestinal infections. These results are coincident with the results obtained for urine stored at 20 °C (Senecal et al., 2018), were no risk to health was found. Transmitted diseases via urine are considered a limited risk in tempered countries. The measured amount of nitrogen in SHU was 4.37 g L^{-1}, similar to 4.03 g L^{-1} in Bolivian study (Mammani et al., 2015). Water measurements show this element, in general, is adequate for plant irrigation. Electric conductivity (EC), which is a good proxy of water salinity, is equal to 1.25 dSm^{-1}. Indicating there is slight to moderate restriction of use, according to the recommended levels ($0.7 < EC < 3.0$) from FAO Soils Bulletin 10 (FAO, 1970). Most plants are sensitive to sodium and chloride irrigation. Ions results were Na^{+1} = 94.3 mg L^{-1}~ 4.1 mmol L^{-1}. Meaning a slight to moderate degree of restriction of use as irrigation water, while in case of Cl^{-1} = 84.0 mg L^{-1} ~ 2.4 mmol L^{-1} indicate none degree of restriction on use. Worth noting that no health risk elements were found in water samples.

Table 2. Summary of resulting parameters measured in sampling elements (urine, farm soil, water, and plant) and their corresponding technique

Parameter	Element					
	Units	Method	Urine	Farm Soil	Water	Plant(2)
pH		NMX-AA-008	8.94	8.28	7.10	
Electric Conductivity	**	Conductometer	31.2		1.25	
Calcium (Ca^{2+})	*	EDTA method	289.9	280.54	130.4	
Magnesium (Mg^{2+})	*	EDTA method	50.7		26.0	
Sodium (Na^{1+})	*	Flamometry	1400.0		94.3	
Potassium (K^{1+})	*	Flamometry	1860.0		13.4	
Sulfate (SO_4^{-2})	*	NMX-AA-074	710.0		232.0	
Phosphates (PO_4^{-3})	*	NMX-AA-029	180.9		2.6	
Carbonates (CO_3^{-2})	*	Volumetry	576.0		25.6	
Bicarbonates (HCO_3^{-1})	*	Volumetry	90.0		366.0	
Chlorides (Cl^{-1})	*	NMX-AA-073	367.5	53.17	84.0	
Fe	*	Atomic Absorption	8.9	54411.5		
Cu	*	Atomic Absorption	0.0	31.6		
Mn	*	Atomic Absorption	2.4	326.5		
Zn	*	Atomic Absorption	0.1	81.6		
Coliforms		NOM112-SSA1-94	(-)	(-)	(-)	(-)
AMB		NOM092-SSA1-94	(-)	(-)	(-)	(-)
Salmonella		NOM114-SSA1-94	(-)	(-)	(-)	(-)
Molds and yeast		NOM111-SSA1-94	(-)	(-)	(-)	(-)

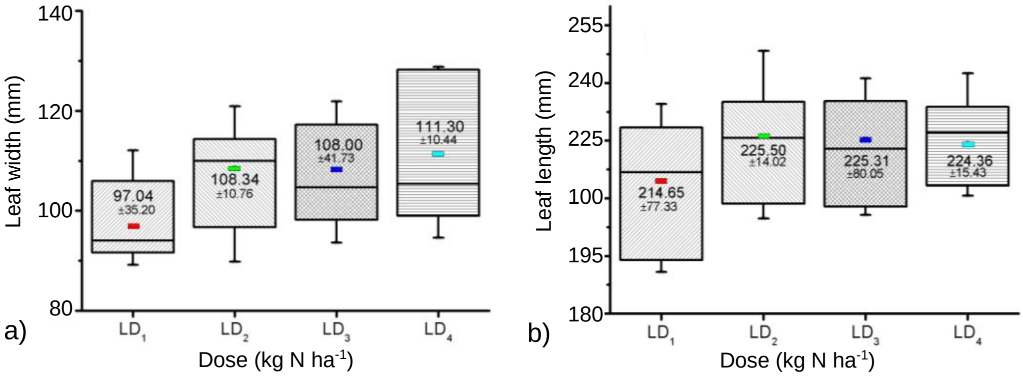
(1) farm soil sample measurements; (2) both lettuce and beets show the same results; AMB = Aerobic Mesophilic Bacteria, and (-) stands for negative; Units equivalences: * (mg L^{-1}), ** (dS m^{-1}).

Lettuce

Lettuce dose-response results of leaf dimensions (width and length) and the plant dry weight are shown in Fig. 2. It is observed that none of these variables has a significant increase with doses. We removed two data points from Fig. 2 these were found in LD_1 and LD_2 treatments, and both correspond to plants that died. The analysis of the dry weight of the plant, Fig. 2, c, shows that the variation of the measurements increases as the dose increase, and we cannot detect a significative change in this variable with the increase in dose. From the analysis of variance, *p*-values of 0.308, 0.412 and, 0.258 were obtained, these correspond to the width, length and dry weight of the plant respectively. This means that there is no significant influence of dose on each one of the response variables for the lettuce.

Beets

In contrast to the development of lettuce under SHU, in case of beet fertilization with SHU, we can observe from Fig. 3, that each dose benefits the plant development. Mainly, the fourth dose (BD_4) generates a substantial increase in the dry weight of the plant. However, at the same time, it is to remark that the growth of the plant is inhibited for the higher concentration dose (e.g., BD_5). To interpret the results more transparent, we have removed one data point, this accounts for BD_3 treatment, and corresponds to died plant. The results of the statistical analysis show that the BD_4 dose significantly affects the dry weight of the plant (p-value = 1.99×10^{-4}). Moreover, also Tukey test results show, from the mean values of treatments, two groups, G_1 and G_2, which means that there is a significative difference between BD_4 treatment and the rest of the treatments, as seen in Fig. 3.



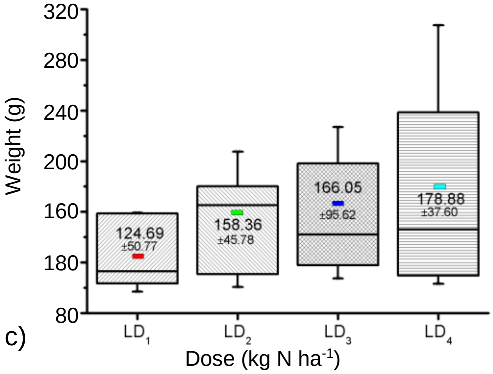


Figure 2. Variation of mean (a) width, (b) length, and (c) dry weight as a function of the supplied dose of SHU in 32 units of Lettuce plants cultivated in pots filled with farm soil substrate. Each box displays the mean value and its corresponding standard deviation. A horizontal line represents the median, and the whiskers correspond to the minimum and maximum values, respectively.

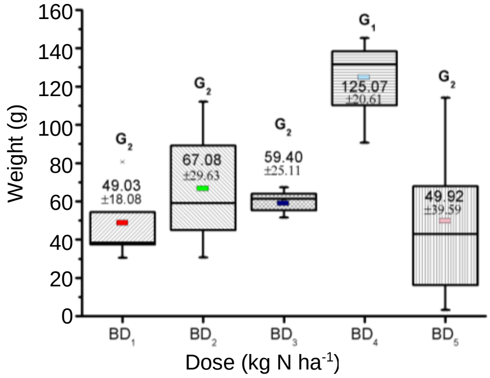
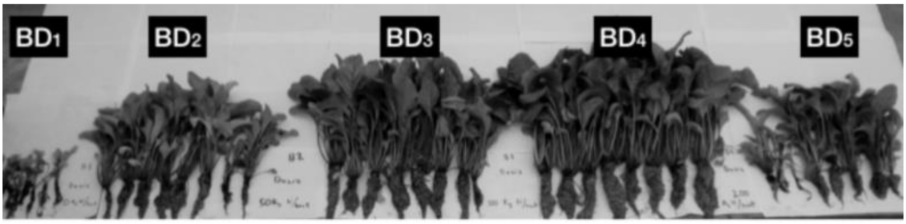


Figure 3. Variation of the mean dry weight of beets (30 units) cultivated in pots filled with farm soil as a function of the supplied dose of SHU. The mean dry weight of beets (filled squares), standard deviation, median (horizontal line), and their Tukey group (G_i) are shown for each dose.

Fig. 4, a shows the development of the beets under different treatments using vermiculite as a substrate. We could appreciate that BD_4 treatment provides the best yield. Fig. 4. shows the corresponding mean dry weight of beets at different doses of SHU. From BD_1 up to BD_4 dose, an increase in the dry weight of beets was shown, similar to Mnkeni et al., (2008), and also agrees that after BD_4 dose, the growth of the plant is inhibited. The maximum mean dry weight (123.52 ± 21.14 g) for beets cultivated with vermiculite differs a 2% from the maximum mean dry weight (125.07 ± 20.06 g) for beets grown with farm soil. The difference could be explained by the farm soil nutrients, absent in vermiculite. As we already mentioned, the farm soil corresponds to a region where the regosols are the characteristic soil type, deficient in nutrients, and high in calcium.

a)



b)

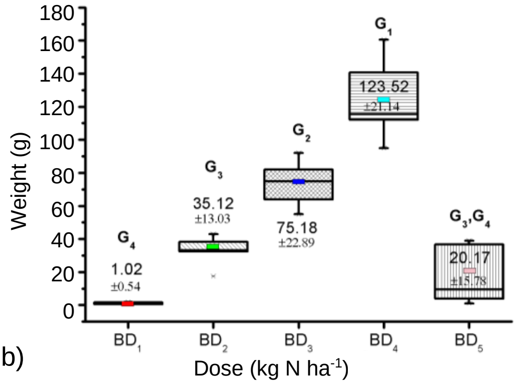


Figure 4. Shows (40 units) beets (a) development and their corresponding (b) mean dry weight at different doses of SHU using vermiculite as substrate. The mean dry-weight of beets (filled squares), standard deviation, median (horizontal line) and their Tukey group (G_i) are shown for each dose.

Statistical analysis of the mean dry weight of beets confirms that each dose (except for BD_5) significantly affects the dry weight of the plant. The calculated *p*-value = 1.45×10^{-15} is eleven orders of magnitude smaller than the corresponding *p*-value for farm soil. This fact, confirms our experimental hypothesis about the vermiculite allows us to study the potential impact SHU supply without considering the soil nutrients. Fig. 4, b shows the mean dry weight of beets at different doses of SHU, and using vermiculite as a substrate. A filled square represents the mean dry weight of beets. And with the aim of more relaxed reading, the numerical value and the standard deviation were added to the side. The corresponding Tukey group (G_i) for each dose is also shown. Tukey test enables us to detect four groups (G_1, G_2, G_3, G_4). Except for the higher dose (BD_5), which shows the coupling of two groups (G_3, G_4) and reduces the development of the plant, all others contain only one group.

In summary, we found that lettuce yield was not benefited under any treatment. While for beet, it was observed that increasing the dose (up to a specific critical value) increases the dry weight of the plant. This difference could be explained since the plants, with different physiology, respond differently to certain factors, specifically to the salinity of the soil, and this, in turn, could help to explain the observed differences. Lettuce, is sensitive to salinity (Ayers et al., 1951; Osawa, 1965; Bernstein et al., 1974), while the beet is tolerant (Bower et al., 1954). Finally, the extrapolation of these results to the region, or over the world, should be taken with cautions. Since soils can be different and characteristics of human urine in terms of the critical nutrients can vary, place to place. The variation of nutrient contents in human urine is attributable to the environmental conditions, physical activity, as well as the diet of the urine donor (Rose et al., 2015). Studies in Finland (Pradhan et al., 2007) and Africa (Mnkeni et al., 2008) report 8.3 g N L^{-1} and 7.4 g N L^{-1} respectively, while in Bolivian study (Mamani et al., 2015) 4.2 g N L^{-1}, similar than our urine measurements (4.37 g N L^{-1}), in contrast, Sweden studies (Kirchmann & Pettersson, 1995) reported 1.79 g L^{-1} to 2.9 g N L^{-1}.

CONCLUSIONS

Based on a review of literature, the lettuce and beets in pots were fertilized with SHU doses. Results show that SHU as fertilizer at the 120 kg N ha^{-1} dose increases the weight of the beet plant and that higher doses inhibit the growth of the plant. The results on the dry weight of beet with both farm soil and vermiculite, allow us to establish with accuracy that the SHU correlates with the growth of the beet plant. In contrast, lettuce did not experience significant changes when fertilized with SHU. We also verify that our experimental samples of farm soil contribute slightly to the vegetative development of the plant when used in combination with the SHU. In other words, the only use of SHU does not maximize beet growth. The farm soil composition improved the dry weight of the plant by 2%; the small difference is due to the lack of farm soil nutrients in regosols founded in the region. However, we could speculate that the farm soil composition considered represents a promising substrate. When, compared with other results (Mnkeni et al., 2008), that uses similar doses of nitrogen on beets, they reported four times lower dry weight of beets.

Therefore, we can carry out the plantation on a larger scale for the production of beets at the local level, on the farm soil, under the explained warnings. In this regard, once the appropriate dose for the beet was identified. We proceed to estimate the possible economic benefits of using human urine as a fertilizer instead of commercial fertilizer, resulting in saving about 780 USD $yr^{-1}ha^{-1}$. This number is a hopeful result for families in peripheral zones of Puebla. However, the urine required to fertilize this same area requires the daily contribution of 50 people, ~12–13 families, and leads to other problems of logistics, distribution, storage, and sanitization, that need further research. Besides, beets are close to the ground and, therefore, insects, and microbial contamination can play an important role. In this regard, our research group is conducting more studies to include these aspects.

ACKNOWLEDGEMENTS. Thanks are due to all members of our academic body DIBACC-321 for enriching this work with their comments, and the grant 00487 VIEP 2019 for the project 'Estimación de contaminantes ($PM_{2.5}$) a partir de datos satelitales y el studio de la orina humana como fertilizante para mejorar el rendimiento de cultivos'.

REFERENCES

Andersson, E. 2015. Turning waste into value: Using human urine to enrich soils for sustainable food production in Uganda. *Journal of Cleaner Production* **96**, 290–298. https://doi.org/10.1016/j.jclepro.2014.01.070

Ayers, A.D., Wadleigh, C.H. & Bernstein, L. 1951. Salt tolerance of six varieties of lettuce. *Proc. Am. Soc. Hort. Sci*. **57**, 237–242.

Batjes, N.H. 1996. Total carbon and nitrogen in the soils of the world. *European Journal of Soil Science* **47**(2), 151–163. https://doi.org/10.1111/j.1365-2389.1996.tb01386.x

Bernstein, L., Francois, L.E. & Clark, R.A. 1974. Interactive effects of salinity and fertility on yields of grains and vegetables. *Agron. J.* **66**, 412–421.

Bibi, S., Saifullah, S., Naeem, A. & Dahlawi, S. 2016. Environmental impacts of nitrogen use in agriculture, nitrate leaching and mitigation strategies. In *Soil Science: Agricultural and Environmental Prospectives*, pp. 131–157. https://doi.org/10.1007/978-3-319-34451-5_6

Bischel, H.N., Özel Duygan, B.D., Strande, L., McArdell, C.S., Udert, K.M. & Kohn, T. 2015. Pathogens and pharmaceuticals in source-separated urine in eThekwini, South Africa. *Water Research* **85**, 57–65. https://doi.org/10.1016/j.watres.2015.08.022

Bower, C.A., Moodie, C.D., Orth, P. & Gschwend, F.B. 1954. Correlation of sugar beet yields with chemical properties of a saline-alkali soil. *Soil Sci.* **77**, 443–451.

Colleen, S., Myhrberg, H. & Mårdh, P.A. 1980. Bacterial colonization of human urethral mucosa: I. scanning electron microscopy. *Scandinavian Journal of Urology and Nephrology* **14**(1), 9–15. https://doi.org/10.3109/00365598009181183

Duniya, V.S. 2018. Effect of male and female urine on growth and phytochemical constituents of Zea Mays. *Open Access Journal of Science*. https://doi.org/10.15406/oajs.2018.02.00105

FAO. 1970. Physical and Chemical Methods of Soil and Water Analysis. *Soil Bulletin* (Vol. **10**). *Food and Agriculture Organization of the United Nations*. Retrieved from https://books.google.com.mx/books?id=cXYbnQEACAAJ

FAO. 2015. World reference base for soil resources 2014. International soil classification system. *World Soil Resources Reports* No. **106**.

Heinonen-Tanski, H. & Van Wijk-Sijbesma, C. 2005. Human excreta for plant production. *Bioresource Technology* **96**(4), 403–411. https://doi.org/10.1016/j.biortech.2003.10.036

Höglund, C. 2001. *Evaluation of Microbial Health Risks Associated with the Reuse of Source-Separated Human Urine*. Doctoral Thesis. Kungl Tekniska Högskoland. Retrieved from http://www.diva-portal.org/smash/record.jsf?pid=diva2%3A8844&dswid=3627

Kirchmann, H. & Pettersson, S. 1995. Human urine - Chemical composition and fertilizer use efficiency. *Fertilizer Research* **40**, 149–154.

Köppen, W. 1918. Klassifikation der Klimate nach Temperatur, Niederschlag und Jahresablauf. Petermanns Geogr. Mitt. **64**, 193–203, 243–248.

Larsen, T.A. & Gujer, W. 1997. The concept of sustainable urban water management. *Water Science and Technology* **35**(9), 3–10. https://doi.org/10.1016/S0273-1223(97)00179-0

Liu, C.W., Sung, Y., Chen, B.C. & Lai, H.Y. 2014. Effects of nitrogen fertilizers on the growth and nitrate content of lettuce (Lactuca sativa L.). *International Journal of Environmental Research and Public Health* **11**(4), 4427–4440. https://doi.org/10.3390/ijerph110404427

Makaya, J.M., Savadogo, A., Somda, M.K., Bour, J.-B., Barro, N. & Traoré, A.S. 2014. Quality of Human Urine Used as Fertilizer: Case of an Ecological Sanitation System in Ouagadougou Peri-Urban Areas-Burkina Faso. *Journal of Environmental Protection* **05**(06), 467–474. https://doi.org/10.4236/jep.2014.56049

Mamani Mamani, V., Loza Murguía, M., Coronel-Quispe, L., Sainz Mendoza, H., Paye-Huaranca, V. & Coronel, F. 2015. Uso de la orina humana como fertilizante en la producción de lechuga Waldmann green (Lactuca sativa L.). *Journal of the Selva Andina Biosphere* **3**(1), 24–38.

Maurer, M., Pronk, W. & Larsen, T.A. 2006. Treatment processes for source-separated urine. *Water Research*. https://doi.org/10.1016/j.watres.2006.07.012

Martínez-Villegas, N. 2007. An overview of different soil classification systems used in Mexico. *Terra Latino Americana* **25**(4), 357–362. Retrieved from http://www.redalyc.org/pdf/573/57315558003.pdf

Mnkeni, P.N.S., Kutu, F.R., Muchaonyerwa, P. & Austin, L.M. 2008. Evaluation of human urine as a source of nutrients for selected vegetables and maize under tunnel house conditions in the Eastern Cape, South Africa. *Waste Management and Research* **26**(2), 132–139. https://doi.org/10.1177/0734242X07079179

Nagy, J. & Zseni, A. 2017. Human urine as an efficient fertilizer product in agriculture. *Agronomy Research* **15**(2), 490–500.

Osawa, T. 1965. Studies on the salt tolerance of vegetable crops with special reference to mineral nutrition. *Bull Univ. Osaka Prefecture*, Series B, Osaka, Jap., **16**, 13–57.

Pavlou, G.C., Ehaliotis, C.D. & Kavvadias, V.A. 2007. Effect of organic and inorganic fertilizers applied during successive crop seasons on growth and nitrate accumulation in lettuce. *Scientia Horticulturae* **111**(4), 319–325. https://doi.org/10.1016/j.scienta.2006.11.003

Pradhan, S.K., Nerg, A.M., Sjöblom, A., Holopainen, J.K. & Heinonen-Tanski, H. 2007. Use of human urine fertilizer in cultivation of cabbage (Brassica oleracea) - Impacts on chemical, microbial, and flavor quality. *Journal of Agricultural and Food Chemistry* **55**(21), 8657–8663. https://doi.org/10.1021/jf0717891

Pradhan, S.K., Holopainen, J.K., Weisell, J. & Heinonen-Tanski, H. 2010. Human Urine and Wood Ash as Plant Nutrients for Red Beet (*Beta vulgaris*) Cultivation: Impacts on Yield Quality. *Journal of Agricultural and Food Chemistry* **58**(3), 2034–2039. https://doi.org/10.1021/jf9029157

Premanandarajah, P. 2017. Selection of Best Combination of Nutrient Sources for Efficient Utilization of Nitrogen in Sandy Regosols. *International Journal of Research in Environmental Science* **3**(4), 29–32.

Rose, C., Parker, A., Jefferson, B. & Cartmell, E. 2015. The Characterization of Faeces and Urine: A Review of the Literature to Inform Advanced Treatment Technology, *Critical Reviews in Environmental Science and Technology* **45**(17), 1827–1879. doi:10.1080/10643389.2014.1000761

Richert, A., Gensch, R., Jönsson, H., Stenström, T.-A. & Dagerskog, L. 2010. *Practical Guidance on the Use of Urine in Crop Production*. *Stockholm Environment Institute (SEI)*. Retrieved from http://www.ecosanres.org/pdf_files/ESR2010-1-PracticalGuidanceOnTheUseOfUrineInCropProduction.pdf

Senecal, J., Nordin, A., Simha, P. & Vinnerås, B. 2018. Hygiene aspect of treating human urine by alkaline dehydration. *Water Research* **144**, 474–481. https://doi.org/10.1016/J.WATRES.2018.07.030

Taylor, C.B. 1997. Plant vegetative development: From seed and embryo to shoot and root. *Plant Cell* **9**(7), 981–988. https://doi.org/10.1105/tpc.9.7.981

Thornton, L.A., Burchell, R.K., Burton, S.E., Lopez-Villalobos, N., Pereira, D., MacEwan, I., … & Gal, A. 2018. The Effect of Urine Concentration and pH on the Growth of Escherichia Coli in Canine Urine In Vitro. *Journal of Veterinary Internal Medicine* **32**(2), 752–756. https://doi.org/10.1111/jvim.15045

Tortora, G.J., Funke, B.R. & Case, C.L. 1989. *Microbiology: an introduction*. Benjamin/Cummings Pub. Co. 8th Ed., 958 pp.

van der Ploeg, R.R., Schweigert, R.P. & Bachmann, R.J. 2001. Use and Misuse of Nitrogen in Agriculture: The German Story. *The Scientific World Journal* **1**, 737–744. https://doi.org/10.1100/tsw.2001.263

Vatansever, R., Ozyigit, I.I. & Filiz, E. 2017. Essential and Beneficial Trace Elements in Plants, and Their Transport in Roots: a Review. *Applied Biochemistry and Biotechnology* **181**(1), 464–482. https://doi.org/10.1007/s12010-016-2224-3

Vinnerås, B., Nordin, A., Niwagaba, C. & Nyberg, K. 2008. Inactivation of bacteria and viruses in human urine depending on temperature and dilution rate. *Water Research* **42**(15), 4067–4074. https://doi.org/10.1016/j.watres.2008.06.014

von Münch, E. & Winker, M. 2011. *Technology review of urine diversion components: Overview of urine diversion components such as waterless urinals, urine diversion toilets, urine storage and reuse systems*. *Deutsche Gesellschaft für Internationale Zusammenarbeit GmbH*. Retrieved from http://www.giz.de/Themen/en/8524.htm

White, P.J. & Brown, P.H. 2010. Plant nutrition for sustainable development and global health. *Annals of Botany* **105**(7), 1073–1080. https://doi.org/10.1093/aob/mcq085

Wielemaker, R.C., Weijma, J. & Zeeman, G. 2018. Harvest to harvest: Recovering nutrients with New Sanitation systems for reuse in Urban Agriculture. *Resources, Conservation and Recycling* **128**, 426–437. https://doi.org/10.1016/j.resconrec.2016.09.015

Wohlsager, S., Clemens, J., Nguyet, P.T., Rechenburg, A. & Arnold, U. 2010. Urine - A Valuable Fertilizer with Low Risk after Storage in the Tropics. *Water Environment Research* **82**(9), 840–847. https://doi.org/10.2175/106143010x12609736967125

Agronomy Research **17**(6), 2233–2241, 2019
https://doi.org/10.15159/AR.19.217

Some rheological properties of new and used mineral lubricant and biolubricant for tractors

J. Csillag[1], A. Petrović[1,*], V. Vozárová[1], J. Kosiba[2] and R. Majdan[2]

[1]Slovak University of Agriculture in Nitra, Faculty of Engineering, Department of Physics, Trieda Andreja Hlinku 2, SK949 01 Nitra, Slovakia
[2]Slovak University of Agriculture in Nitra, Faculty of Engineering, Department of Transport and Handling, Trieda Andreja Hlinku 2, SK949 01 Nitra, Slovakia
[*]Correspondence: ana.petrovic@uniag.sk

Abstract. It is important to know physical characteristics of lubricating oils to ensure the highest reliability for operation of device. The use of ecological lubricants depends on their characteristics; the most important are density and viscosity, protection against wear and tear, corrosion resistance etc. The objective of this work was to find changes of the rheological properties of the synthetic oil and bio lubricant. We compared two different oils in our measurements. One sample was synthetic oil and the other was mineral oil (bio lubricant). Both oils are universal oils for tractors. Further, comparison of new and used sample after million cycles was performed. The density and the dynamic viscosity show strong exponentially decreasing dependence. With the increasing temperature, values of the both properties, decreased. It can be also observed that used samples have lower viscosity and density. The results presented in this article can be important when putting ecological lubricants into operation.

Key words: dynamic viscosity, density, ecological oil ertto, fluidity, kinematic viscosity, mineral oil ultra.

INTRODUCTION

Environmentally friendly hydraulic fluids have specific characteristics determined by their readily biodegradable. The main conditions required for biodegradable lubricants imply that they must be readily biodegradable and they cannot have toxic effect on organisms in the environment where they have penetrated. The biggest risk arises when the hydraulic fluid gets into surface waters and ground waters (Rusnák et al., 2009). One of the ways to reduce the adverse effects on the ecosystem caused by the use of lubricants is to replace petroleum base oil with biodegradable products. The aim of the article is to compare rheological characteristics (density and viscosity) of mineral oil with ecological oil. We compared new samples with samples after accelerated test of million cycles of pressure stress.

‘Majdan & Tkáč (2008) dealt with comparison of ecological fluid Ertto and mineral oil Ultra, too.’ They studied flow characteristics and stated that ecological fluid does not have negative impact on lifetime of pump. ‘Majdan et al. (2016) did research on a universally useful filtration system for increasing the cleanliness level of biodegradable oils.' 'Bell (1993) states that the oil must remain fluid under all conditions to perform its functions, ideally maintaining the right physical properties to perform them efficiently'. The viscosity of the oil plays a predominant role. High viscosity oils would be favored simply to prevent wear. Many other publications, e.g. Guo et al. (2007), Mang & Dresel (2007) and Albertson et al. (2008) have suggested that density and dynamic viscosity show high temperature dependence while measuring, particularly when increasing the temperature, the value of density and dynamic viscosity decreased.' 'Sejkorová et al. (2017) presented measurement of dependence of viscosity on wear and tear (mileage) in their article, while presenting graphical dependencies of decreasing value of viscosity with the increasing wear and tear.' 'Nedić et al. (2009) observed the value of viscosity up to prescribed interval of substitution in their article.' 'Some other parameters, which define operating instructions of ecological oils were studied in Tóth et al. (2014).' 'According to Sejkorová et al. (2017) one of the reasons for decreasing viscosity could be intrusion of impurities from circulatory system into oil.' The low viscosity of oil means too thin film, which could lead to bigger wear and tear of device. It means that lower viscosity causes bigger wear and tear. 'Tkáč et al. (2017) consider oils as a media that helps to obtain information on processes and changes in the systems they lubricate.'

MATERIAL AND METHODS

We compared two different oils in our measurements. One sample was synthetic oil and the other was biolubricant. Both oils are universal oils for tractors. They are used for greasing of transmissions, hydraulic circuits and wet brakes of agricultural and construction machinery. Mol Traktol Ertto (Environmentally Responsible Tractor Transmission Oil) is an ecological oil which is biodegradable according to CEC L-33-A-93 (28 days) almost 91%.

Production of oil is based on the basis of vegetable oil with addition of special types of additives. Oil is especially suitable for machines working near water sources or the forest environment. Thanks to high rate of biodegradability, oil MOL Traktol NH Ultra can be categorized as mineral oil of type HV. It reaches almost 45% of biodegradability according to CEC L-33-A-93 (28 days).

Test was realized on a special test device according to the standard STN 11 9287 on Department of Transport and Handling, SUA in Nitra. Hydraulic pump was loaded with pressure changing cyclically from 0.1 MPa to the nominal pressure of the hydraulic pump 20 MPa during the test. Mainly these pressure impacts burdens hydraulic system of tractor the most. That is one of the main reasons of choosing this type of straining in laboratory conditions (Tkáč et al., 2010; Majdan et al., 2011; Tkáč et al., 2014).

The measurement of density was carried out on densimeter Mettler Toledo DM40. The principle of measurement of density is based on electromagnetic induced oscillation of U–shaped glass tube. Dynamic viscosity was measured on viscometer DV2T fy Brookfield. The both measuring devices are part of the Agrobiotech Research Center in Nitra, Slovakia. The principle of measurement is based on measurement of torque of spindle rotating in the sample at constant speed.

Dynamic viscosity derived from Newton's law is characterized by a relationship:

$$\tau = \eta \; grad \; v \tag{1}$$

where $grad \; v = \frac{dv}{dh}$ – the size of velocity gradient (s^{-1}); τ – shear stress (Pa); η – dynamic viscosity (Pa s).

The basic unit of dynamic viscosity is Pa s. More commonly, a thousand times smaller unit mPa s is used (Krempaský, 1982; Ruban & Gajjar, 2014).

The temperature effect on viscosity can be described by an Arrhenius type equation:

$$\eta = \eta_0 e^{-\frac{E_A}{RT}} \tag{2}$$

where η – dynamic viscosity (Pa s); η_o – the reference value of dynamic viscosity (Pa s); E_A – activation energy ($J \, mol^{-1}$); R – gas constant ($8.314472 \, J \, K^{-1} \, mol^{-1}$); T – thermodynamic temperature (K).

Many authors (Munson et al., 1994; Hlaváč et al., 2016) explain, that the temperature dependence of viscosity can also be explained by cohesive forces between molecules. With increasing temperature, these cohesive forces between molecules decrease and flow becomes freer. As a result, viscosity of liquids decreases with increasing temperature. The fluidity of liquids in liquid state can be explained by relatively weak forces of mutual activity of molecules and their high movability. The fluidity of different liquids is different and it equals to reciprocal of dynamic viscosity, with basic unit $Pa^{-1} \, s^{-1}$.

$$\varphi = \frac{1}{\eta} \tag{3}$$

Kinematic viscosity can be defined as quotient of dynamic viscosity and density of liquids if they are measured at the same temperature:

$$v = \frac{\eta}{\rho} \tag{4}$$

where η is dynamic viscosty in Pa s and ρ is density in $kg \, m^{-3}$. The basic unit of kinematic viscosity is $m^2 \, s^{-1}$, but smaller unit $mm^2 \, s^{-1}$ is usually used.

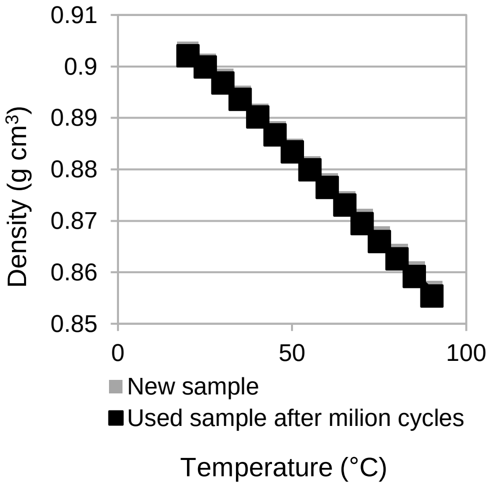
The fluidity is ability of matters to flow. Parts of liquid matters can move easily towards one another, because the particles are not bounded in fixed positions. The measure of fluidity is expressed by viscosity. It is defined as reciprocal of dynamic viscosity Eq. (3).

RESULTS AND DISCUSSION

The density measurements were made in interval from 20 °C to 90 °C. From the measured data, we created graphical dependencies of the density from temperature for all samples.

On Fig. 1 it is clear that the new sample and the cyclically worn sample have very similar density values. Decrease in density of both samples is evident. After this comparison, we can conclude that the process of wear of the hydraulic pump occurred, resulting in the lowest density values in used Ertto sample.

Fig. 2 shows that the samples have similar density values. For the sample of one million cycles, the smaller density values were measured - a new sample at 40 °C had a density of 875.5 $kg \, m^{-3}$ and the sample after one million cycles reached a density of 874.2 $kg \, m^{-3}$. The decline is also seen in graphical dependence, but the differences are not significant. It is also noticeable that bigger differences occurred in sample Ultra in higher temperatures.



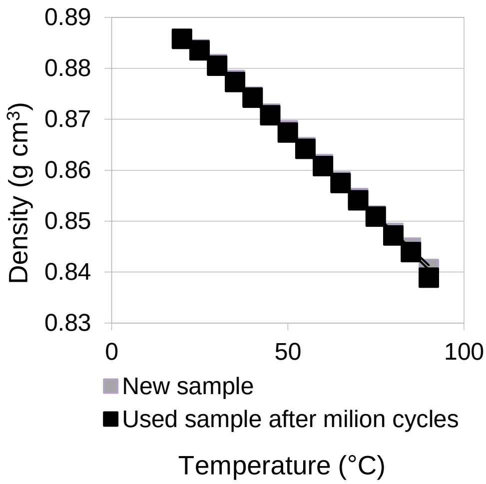


Figure 1. Temperature dependencies of density for samples Ertto.

Figure 2. Temperature dependencies of density for samples Ultra.

In the Table 1, we place exponential regression equations and coefficient of determination R^2. As it can be seen from the dependencies for all samples, the development is characterized by the curve very well. The determination coefficients for all the samples are very high, which also confirms strong exponentially decreasing dependence.

Table 1. The overview of exponential regression equations and coefficients of determination of dependence of density (ρ) on temperature (t) for sample Ertto

	Ertto (Eco)		Ultra (Mineral)	
	New Sample	Used sample after million cycles	New Sample	Used sample after million cycles
Regression equation	ρ = 0.918e$^{-0,0008t}$	ρ = 0.917e$^{-0,0008t}$	ρ = 0.901e$^{-0,0008t}$	ρ = 0.901e$^{-0,0008t}$
Determination coefficient R^2	0.999	0.9988	0.9986	0.9977

From the point of view of physical interpretation, the exponential dependence of the Arrhenius type is better suited. ‘Nešťák (2016) measured the density of ecological lubricants, while the sample he used Mogus Hees 46 had a density of 906.5 kg m^{-3} at 20 °C and at a temperature of 90 °C the density was reduced to 859.9 kg m^{-3}’. The coefficient of determination directly reached 1. To compare our ecological liquid Ertto, which at 20 °C had a density of 902.7 kg m^{-3} and after reaching 90 °C the density dropped to 856.3 kg m^{-3}. Our sample has a determination coefficient of 0.999. Decrease has been almost linear in some parts of the graphical dependency. When comparing with the values given by the manufacturer, small differences were found, which may be due to measurement inaccuracy, aging, or sample storage.

The viscosity measurements were made in interval from 20 °C to 90 °C. The data of viscosity at temperature of 95 °C and 100 °C were calculated from regression equation. From the data, we created graphical dependencies of the dynamic viscosity on temperature for all samples.

Temperature dependencies of dynamic viscosity for both samples Ertto are shown in Figs 3, 4. It is possible to observe that dynamic viscosity of samples is decreasing exponentially with increasing temperature, what was expected and what corresponds with conclusions reported in literature (Trávníček et al., 2013; Hlaváč et al., 2014; Vozárová et al., 2015). We also noticed a difference between new and the used sample. The sample, which has been used already for one million cycles, has lower viscosity in all temperature range. Further, differences between viscosities at lower temperatures are greater and also more spread. Which means that it is not enough to measure this physical property just at one temperature. Wider range is necessary to know better the sample behavior.

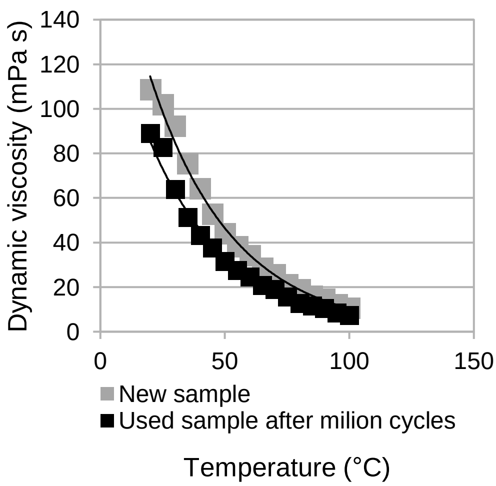


Figure 3. Temperature dependencies of dynamic viscosity for samples Ertto.

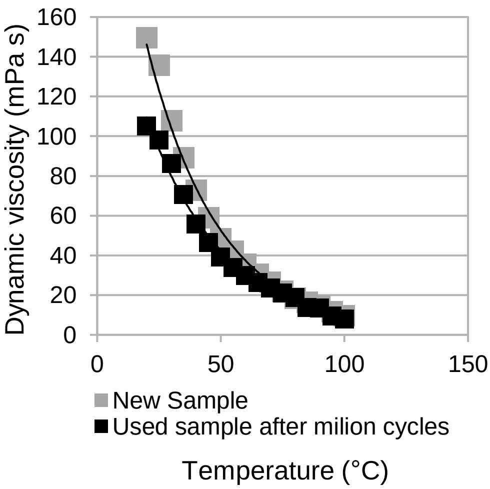


Figure 4. Temperature dependencies of dynamic viscosity for samples Ultra.

When it comes to comparison between mineral oil - Ultra and biological one - Ertto, we also noticed differences. First characteristic is viscosity at lower temperatures. Where, in case of sample Ultra, we can observe much higher viscosity than particular one of sample Ertto. But with the increase of temperature, the values of those two samples, are much more similar. And observing Figs 3, 4, we can conclude that difference between new sample Ultra and used sample Ultra are much bigger, than new and used sample Ertto.

The coefficients of regression equations and coefficients of determination are summarised in the Table 2. The determination coefficients for all the samples are very high, which also confirms strong exponentially decreasing dependence. The progress can be described by a decreasing exponential function, which is in accordance with Arrhenius equation.

Table 2. Overview of exponential regression equations and coefficients of determination of dependence of dynamic viscosity (η) on temperature (t) for the sample Ertto and Ultra

	Ertto		Ultra	
	New Sample	Used sample after million cycles	New Sample	Used sample after million cycles
Regression equation	$\eta = 211.0e^{-0.03t}$	$\eta = 159.8e^{-0.03t}$	$\eta = 291.9e^{-0.03t}$	$\eta = 214.4e^{-0.03t}$
Determination coefficient R^2	0.996	0.994	0.995	0.996

The comparison of mineral oil and ecological one is shown in the Table 3. Bigger difference between the new sample and the sample after accelerated test was in ecological fluid.

Table 3. The comparison of dynamic viscosity of ecological and mineral oil at temperature of 100 °C

	Mineral Oil Ultra	Ecological fluid Ertto
New Sample	9.74 mPa s	10.5 mPa s
Used sample after 10^6 cycles	7.9 mPa s	7.2 mPa s

As the literature mostly report kinematic viscosity at 40 °C, thus we calculated it according to the Eq. (4), namely the measured dynamic viscosity divided by the measured density. Graphical dependences of the kinematic viscosity on temperature were created for all samples. We can observe from Fig. 5 and Fig. 6 that the kinematic viscosity of samples is decreasing with increasing temperature.

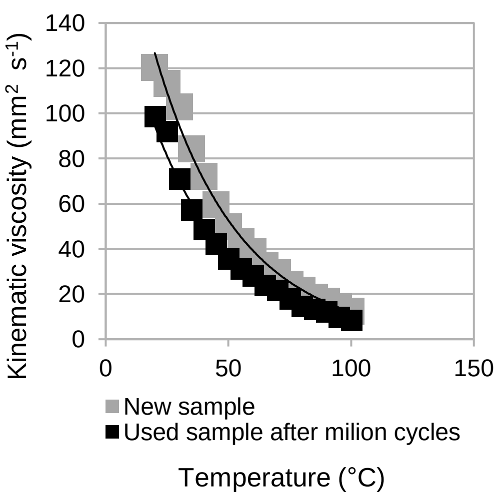


Figure 5. Temperature dependencies of kinematic viscosity for samples Ertto.

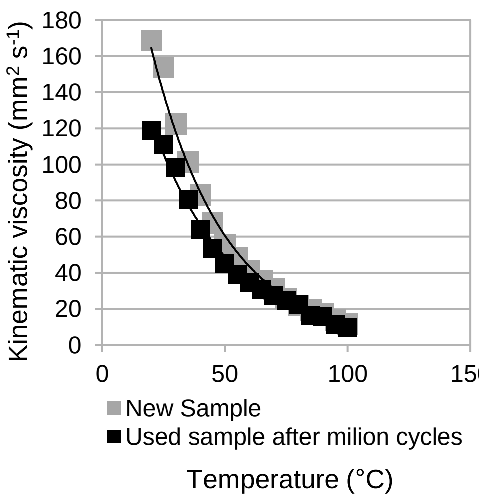


Figure 6. Temperature dependencies of kinematic viscosity for samples Ultra.

It seems that the values of the kinematic viscosities decreased in cyclically worn Ertto sample. It is also observed that the differences are bigger at lower temperatures. For comparison, we can see that kinematic viscosity of new Ertto at 40 °C has 64.2 $mm^2 s^{-1}$ and cyclically worn sample has 43.33 $mm^2 s^{-1}$. As an indicator of viscosity change we indicate viscosity index (VI). Oils with high viscosity index (> 130) cause less wear. New Ertto sample has VI of 152.849, and the worn one has 128.084. As it is known, the greater the VI, the smaller the change in fluid viscosity. Thus, a lower VI in the used sample, could be assumed.

We can see from graphical dependencies that new sample of Ultra had higher viscosity than the sample after accelerated test. In Fig. 6 is shown that the figure of viscosity changed at low temperatures in a rather wide range. When increasing the temperature, the figure of viscosity for each sample differs in a smaller range. For better understanding, we state for the Ultra new sample at 40 °C 83.332 $mm^2\ s^{-1}$ and for the 1 million cycles sample 63.829 $mm^2\ s^{-1}$. We can conclude that difference between new and worn Ertto sample is bigger than of Ultra ones. It is also confirmed by VI. New Ultra sample as VI of 132.604, and worn one has 129.625. The higher VI, the smaller is the difference between high viscosity and lower viscosity of oil.

The fluidity of compared samples Ertto and Ultra is shown on Figs 7, 8.

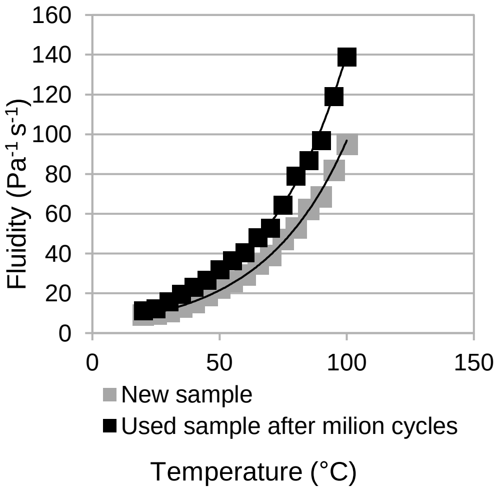


Figure 7. Temperature dependencies of fluidity for samples Ertto.

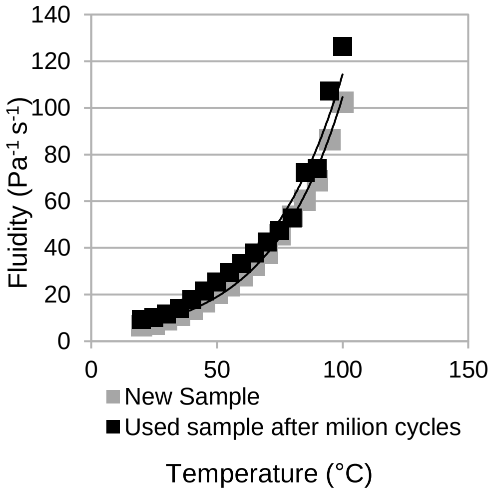


Figure 8. Temperature dependencies of fluidity for samples Ultra.

Differences can be observed from the Fig. 7 at the beginning and at the end of temperature range. Namely, at 20 °C Ertto new sample has fluidity of 9.208 $Pa^{-1}\ s^{-1}$ and used one has 11.24 $Pa^{-1}\ s^{-1}$. But at higher temperatures differences are bigger. When we compare fluidity at 100 °C of new and used sample, we got 95.238 $Pa^{-1}\ s^{-1}$, 138.84 $Pa^{-1}\ s^{-1}$, respectively. So the total difference between samples at 20 °C is 2.032 $Pa^{-1}\ s^{-1}$ and at 100 °C is 43.602 $Pa^{-1}\ s^{-1}$.

Contrary to the previous sample, differences between new and used Ultra sample are not so great. Measured value at 20 °C of the Ultra new sample is 6.68 $Pa^{-1}\ s^{-1}$ and of used one is 9.5057 $Pa^{-1}\ s^{-1}$. Fluidity at 100 °C of the Ultra new sample is 102.631 $Pa^{-1}\ s^{-1}$ and of used one is 126.458 $Pa^{-1}\ s^{-1}$.

We can see from graphical dependencies (Figs 7, 8) that, when heating the compared samples, their fluidity increases. As the samples after a million cycles of accelerated test had lower viscosity in comparison with the new samples, the fluidity of these samples was higher.

CONCLUSION

The results presented in this article can be important when putting ecological lubricants into operation. The use of ecological lubricants depends on their characteristics; the most important are density and viscosity, protection against wear and tear, corrosion resistance etc. It is important to know physical characteristics of lubricating oils to ensure the highest reliability for operation of device. Knowledge of the density and viscosity behaviour of an engine oil as a function of its temperature has a big importance, especially when considering running efficiency and performance of combustion engines. The objective of this work was to find changes of the rheological profile of the mineral oil and bio lubricant. The density and the dynamic viscosity show the significant temperature dependence. With the increasing temperature, the density and the dynamic viscosity decreased. Temperature dependencies of samples of dynamic viscosity had an exponential decreasing shape, which is in accordance with Arrhenius equation.

We have compared ecological oil and mineral oil in our work. At the same time, we have compared new sample with the sample after accelerated test according to the standard STN 11 9287. In both cases (Ertto and Ultra) changes in the color were not measured, only based on the visual, it can be stated that the used sample is darker. Ecological oil Ertto reached higher density than a mineral oil, but the difference between the new sample and the sample after accelerated test was little in both cases. Dependence of viscosity on temperature confirmed decreasing exponential function, while mineral oil had higher value of viscosity. The samples after accelerated test had lower dynamic and also kinematic viscosity than new samples. For fluidity applies that samples after test reached higher values than new samples. Ecological oils in comparison with mineral oils have higher fluidity.

ACKNOWLEDGEMENTS. This work was supported by the project KEGA 017SPU-4/2017 of Ministry of Education, Science, Research, and Sport of the Slovakia and by European Community under project no 26220220180: Building Research Centre 'AgroBioTech'.

REFERENCES

Albertson, W.C., Staley, D.R., Mcdonald, M.M. & Pryor, B.K. 2008. *Engine oil viscosity diagnostic system and methods*. United States Patent 20080223114.

Bell, J.C. 1993. Engine lubricants. *Tribology Series* **26**, 287–301.

Guo, B., Lyons, W. & Ghalambor, A. 2007. *Petroleum production engineering*. United Kingdom: Elsevier Science and Technology Books, 288 pp.

Hlaváč, P., Božiková, M. & Cviklovič, V. 2016. Dynamic Viscosity and Activation Energy of Wort During Fermentation and Storing. *Acta Technologica Agriculturae* **19**(1**)**, 6–9.

Hlaváč, P., Božiková, M. & Presová, R. 2014. Temperature relations of selected engine oils dynamic viscosity. *Acta Technologica Agriculturae* **17**(4), 105–108.

Krempaský, J. 1982. *Physics*. Alfa, Bratislava, 752 pp. (in Slovak).

Majdan, R., Cvíčela, P., Drabant, Š., Tkáč, Z., Kosiba, J. & Abrahám, R. 2011. *Assessment of ecological gear-hydraulic fluids based on operational load tests*. Nitra: SUA, 127 pp. (in Slovak).

Majdan, R. & Tkáč, Z. 2008. *Application of ecological liquids in mobile machinery hydraulic systems*. Nitra: SUA, 119 pp. (in Slovak).

Majdan, R., Tkáč, Z., Abrahám, R., Szabó, M., Halenár, M., Rášo, M. & Ševčík, P. 2016. Proposal for filtration system for biodegradable lubricants in agricultural tractors. *Agronomy Research* **14**(4), 1395–1405.

Mang, T. & Dresel, W. 2007. *Lubricant and Lubrication*. Weinheim: Wilez-Vch GmbH, 850 pp.

Munson, B.R., Young, D.F. & Okiishi, T.H. 1994. *Fundamentals of fluid mechanics*. New York: John Wiley & Sons. 783 pp.

Nedić, B., Perić, S. & Vuruna, M. 2009. Monitoring physical and chemical characteristics oil for lubrication. *Tribology in industry* **31**(3–4), pp. 59–66.

Nešťák, S. 2016. *The use of rheological properties in the evaluation of quality of ecological lubricants*. Nitra: SUA, 60 pp. (in Slovak).

Ruban, A.I. & Gajjar, J.S.B. 2014. *Fluid dynamics*. Oxford: University press, 317 pp.

Rusnák, J., Kadnár, M. & Kučera, M. 2009. *Biodegradable oils and their tribological properties*. Nitra: SUA, 85 pp. (in Slovak).

Sejkorová, M., Hurtová, I., Glos, J. & Pokorný, J. 2017. Definition of a Motor Oil Change Interval for High-Volume Diesel Engines Based on its Current Characteristics Assessment. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis* **65**(2), 481–490.

Tkáč, Z., Čorňák, Š., Cviklovič, V., Kosiba, J., Glos, J., Jablonický, J. & Bernát, R. 2017. Research of Biodegradable Fluid Impacts on Operation of Tractor Hydraulic System. *Acta Technologica Agriculturae* **20**(2), 42–45.

Tkáč, Z., Majdan, R., Drabant, Š., Abrahám, R., Vozárová, V. & Jablonický, J. 2010. *Evaluation of hydraulic fluid properties on the basis of hydraulic pump tests*. Nitra: SUA, 112 pp. (in Slovak).

Tkáč, Z., Majdan, R. & Kosiba, J. 2014. *Research of properties of ecological liquids and new test methods of lubricating oils*. Nitra: SPU, 94 pp. (in Slovak).

Tóth, F., Rusnák, J., Kadnár, M. & Čavojský, P. 2014. Effect of Selected Ecological Lubricants on the Wear of Defined Sliding Bearing. *Acta Technologica Agriculturae* **17**(1), 13–16.

Trávniček, P, Valach, M., Hlaváčová, Z., Mareček, J., Vítěz, T. & Junga, P. 2013. Selected physical properties of liquid biofuels. *Research in Agricultural Engineering* **59**(4), 121–127.

Vozárová, V., Kardjilova, K., Híreš, Ľ., Valach, M. & Wollner, A. 2015. Temperature dependence of dynamic viscosity and DSC analysis of the Plantohyd samples. *Journal of Central European Agriculture* **16**(2), 221–230.

Agronomy Research **17**(6), 2242–2259, 2019
https://doi.org/10.15159/AR.19.221

Typology of small producers in transition to agroecological production

N. Escobar[1,*], N.J. Romero[2] and C.I. Jaramillo[3]

[1]University of Cundinamarca, Faculty of Agricultural Sciences, 18th Diagonal # 20-29, Fusagasuga, Colombia
[2]University of Tolima, Faculty of Veterinary Medicine and Animal Production, Barrio Santa Helena, Ibague, Colombia
[3]Agrosavia. National Research Center of Agriculture, Nataima, Colombia
[*]Correspondence: nataliaescobar@ucundinamarca.edu.co

Abstract. Agroecology is now emerging as the fundamental science to guide the conversion of conventional production systems to more diversified and self-sufficient systems. The agroecological transition is defined as the gradual change that farmers undergo to adapt and move from more conventional towards agroecological farming principles, encompassing technological, societal, institutional and organisational changes in the food system. To analyze a transition process, it is initially necessary to understand how agroecosystems work (their structure and processes), and the different ways human beings intervene an ecosystem in order to transform it for productive purposes.Farm systems typology and classification techniques are used to guide strategic lines of research, sectorial policies, and promote sustainable development in response to farmer's needs. Determining multidimensional classification methods in agricultural systems is necessary, considering both the variables inherent to the production system and those of an external nature that indirectly impact the development and long-term sustainability of production systems. One of the purposes of this research was to characterize agricultural production based on sustainability systems and environmental, social, and economic indicators. The study was carried out based on data collected from 71 farm surveys, considering the social, economic, environmental, and technological dimensions. Multiple correspondence and cluster analysis were done. Three types of production systems were obtained: Group I, organic producers in transition; Group II, conventional producers in transition to organic production; and Group III, conventional producers interested in organic production. Producers need to focus on processes that allow them to improve their skills to develop human talent and social capital in terms of integration, collaborative work, trust, political and cultural capital, so that they can make progress easily and start implementing agroecological, infrastructure, and natural resources management practices, while improving their living standards. The information yielded by a typology process allows for us to know the current state of agricultural production systems based on the implementation of agroecological practices; thus facilitating the preparation and implementation of participatory plans and/or integrative proposals that promote agrofood sustainability.

Key words: sustainability, organic agriculture, rural development.

INTRODUCTION

To cope with the production systems complexity and the agroecological transition (AE), it is necessary to understand how the agroecosystem works. For this, a systemic approach is crucial, where the limits of the system, the components that integrate it, and the interrelations that occur between them are considered. This approach allows to organize the knowledge about the agroecosystem functioning, interpreting the particular properties that emerge from these relationships and those responsible for providing useful ecological services from an agroecological approach Duru & Therond, 2015).

Transforming conventional systems to other ecologically based systems is not a simple and quick task, it requires gradual changes in the ways of managing agroecosystems (Caporal et al., 2009). It is necessary to consider productive, cultural, social, economic and political aspects that demand an integral and systemic view. Therefore, a transition process involves a multitude of foreseen and unforeseen causes and effects, and it is constructed over time. As stated by Gliessman et al. (2007), it implies a change in farmers and consumers' values and way of acting, in their social, productive and natural resource relations, i.e. transition occurs not only on the farm, but also at community level.

Typology has been defined by Daloğlu et al. (2014) as a method to identify production systems diversity by ordering or classifying reality. The term 'typology' is used to define types, analyze a complex reality and order objects that, despite being different, fall under the same type, e.g. a farm. Every system or productive unit is different in both structure and function; these two characteristics will determine the relations of homogeneity or heterogeneity between agroecological production systems (Varela, 2010).

Producers typology can be based on previous studies and information available to territorial entities, seeking a first approach to targeted places. Therefore, field work to be in direct contact with producers is necessary for obtaining information for analysis and draw conclusions to improve region's productive systems (Garcia & Calle, 1998). Typology seeks to group producers under similar management, production, and techniques as some producers are in delimited geographical areas.

For decades, methodologies for agricultural production systems typology have been under development. Valuable experiences on their applications have contributed to the knowledge of the agricultural development dynamics of a region, by analyzing the relationship between the types of farms, and their socioeconomic, physical, and biological environment (Varela, 2010). Typologies are a convenient tool to simplify the diversity of farming systems while effectively describing their heterogeneity (Daloğlu et al., 2014). This instrument is used to provide guidelines for the development of agricultural innovations and to better understand their implications in family behavior of the farmers (Douxchamps et al., 2015; Kuivanen et al., 2016; Contzen & Forney, 2017).

In Latin America, there is a need to know where agroecology is being adopted as a production system. The information yielded by a typology process allows to know the current state of the production systems. Agroecology territories are places where a transition process toward sustainable agriculture and food systems is engaged. Classification studies have also been used to manage specific research and development projects to select target groups and representative farms, among others (Escobar & Berdegue, 1990). These classification methods can be univariate or multivariate. The latter are more common due to the involvement of the systemic approach, by relating variables of the farm system with their surrounding environment (Varela, 2010) which allow the multiple measurements of the individuals studied to be analyzed (Carrillo et al., 2011). Shaner et al. (1982) developed criteria based on a limited number of indicators grounded in climate information and soils, to classify farms according to their area and number of farmers. The aim of this study was to typify agricultural production based on sustainability systems and environmental, social and economic indicators, as well as to identify opportunities and barriers for promoting agroecological transitions.

MATERIALS AND METHODS

This research was conducted in 7 stages: 1. Description of target population. 2. Sample selection and construction of data collection instrument. 3. Information processing (database building, classification and description of variables). 4. Review and selection of variables. 5. Application of multivariate statistical techniques. 6. Determination of types or subsystems. 7. Description of types or groups (Sraïri & Lyoubi, 2003).

Study zone

This study was conducted on producers in the Province of Sumapaz, in the department of Cundinamarca (Colombia) (Fig. 1). Land use in the Sumapaz region is focused on 28.6% pastures, 21.5% secondary forest, 14.8% paramo vegetation, 8.3% stubble, 5.3% grass with stubble, 4.4% natural forest, and 9.3% soils is used for agriculture. It has an average temperature between 18 °C and 24 °C and biannual rainy seasons (March-April and July-August) (Giraldo, 2008).

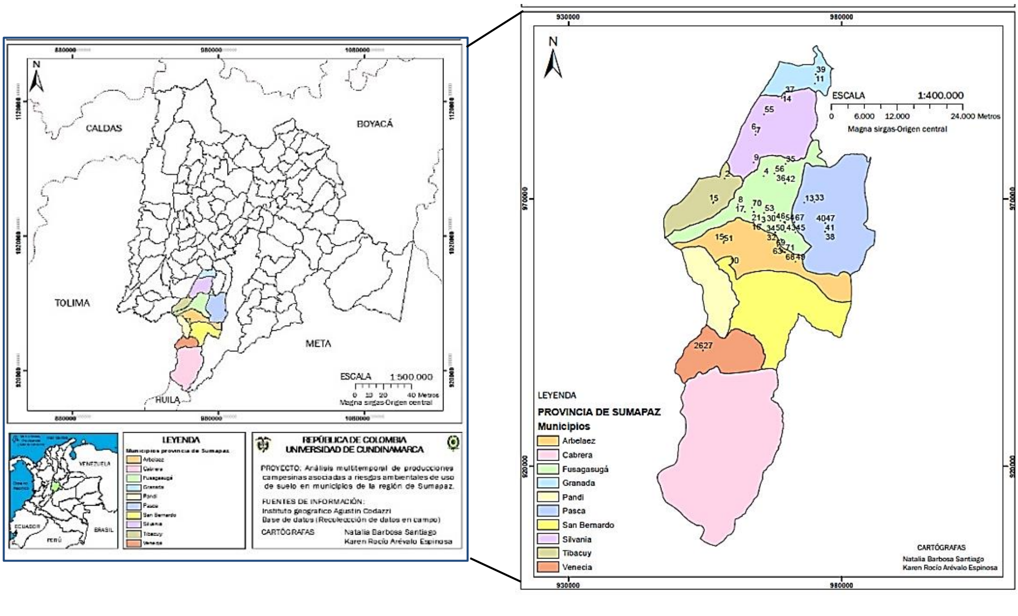


Figure 1. Location of the region of Sumapaz (the 10 municipalities represented in colors) Department of Cundinamarca (left). Location of the 71 respondents (right).

Description of target population, sample selection and collection instrument

Implementing organic agriculture practices within their productive systems was the common activity established. Since no reference framework was available, a snowball sampling was used until some SENA-based organic market producers were reached. They led to other farmers, and a total sample of 71 respondents was achieved (Fig. 1). The surveys, 200 questions each, were conducted from March to July 2015, and focused on for dimensions: Economic, Social, Environmental, and Technological variables. The surveys were carried out directly to producers through visits to the 71 farms.

Statistical analysis

A descriptive analysis to quantify repetition and redundancy observations (Romero, 2009) was carried out, and then the variables grouping the respondents' answers were unified and categorized. Based on this, 36 variables were obtained and narrowed to those being discriminatory so that differences were established. To that end, distribution of variable frequencies was used; e.g., if 98% of producers were farm owners, and only 2% were sharecroppers, such variable would not have contributed much to identify organic producers.

Multiple correspondence analysis (MCA) is an extension of correspondence analysis which allows one to analyze the pattern of relationships of several categorical dependent variables (Abdi et al., 2007). Multivariate techniques of MCA were applied through Burt's method and cluster analysis (CA), using the Euclidean distance and Ward's method (Blazy et al., 2009). The former one, is a factorial technique developed for studying a population of individuals described by a set of categorical variables, each of them with a certain number of categories (Aguirre, 2013), the latter one allows to classify individuals based on homogeneous characteristics to group them.

The *dimdesc* function was used to identify the most correlated variables to the dimensions generated in the MCA, through the coefficient of determination (R^2). Each dimension was analyzed through a factor variance analysis, and an F test was derived to find out if the variable had an impact on the analyzed dimension, and also a T test was performed category by category. In order to compare the variables within the groups resulting from the cluster analysis, contingency charts were made, and the chi-squared test (Chis-q) was used to determine statistical differences between groups with a 99% trust level; then, each cluster was described to characterize the relevant aspects. R 3.2.3v statistical software, using FactomineR, to calculate the results, and Factoextra for graphs visualization, were used.

RESULTS

In order to obtain a typification of the small producers, it is necessary to determine which variables (social, environmental, economic, technological) have the greatest influence on the characterization. Based on the results generated by the multiple correspondence analysis, eight optimal dimensions (axes) were obtained, which combined 74.3% of the cumulative variability. The first and second components explained 35.1% and 12.3% of the variance percentage respectively. The factor map for the distinct categories was obtained by selecting the 40 greatest contributing categories to the first and second components (Fig. 2 – supplementary material).

Component one was characterized by the following set of variables, as follows: produced inputs ($R^2 = 0.5813$, $p = < 0.01$), organic fertilizers ($R^2 = 0.5143$, $p = < 0.01$), infrastructure ($R^2 = 0.4253$, $p = < 0.01$), farm change ($R^2 = 0.4134$, $p = < 0.01$), participation in events ($R^2 = 0.3791$, p = < 0.01), agricultural waste management ($R^2 = 0.3337$, $p = < 0.01$), irrigation ($R^2 = 0.3148$, $p = < 0.01$), agricultural production ($R^2 = 0.2959$, $p = < 0.01$), mother duties ($R^2 = 0.2553$, $p = < 0.01$), product transformation ($R^2 = 0.2787$, $p = < 0.01$), agricultural communication ($R^2 = 0.2698$, $p = < 0.01$), agroecological practices ($R^2 = 0.2431$, $p = < 0.01$), technical assistance service ($R^2 = 0.2730$. p = < 0.01) and being part of an organization ($R^2 = 0.2667$, $p = < 0.01$).

The coordinates of the following categories are positive: '5 or more infrastructures', '3 or more inputs', 'Between 3 and 5 agricultural waste management processes', 'Irrigation', '4 or more events', 'Between 6 and 8 fertilizers', 'Mother with 3 or more duties', 'Between 3 and 5 means of communication', '3 or more livestock systems', '3 or more changes on the farm', 'Decisions made by father and mother',' 3 or more processed products', 'If there are accounting records' and 'If there are associations'; while the following categories are negative: 'There are between 1 and 4 pieces of infrastructures', 'There are no institutions', 'Less than two agricultural waste management processes', 'No irrigation systems', 'No changes on the farm', 'Less than 7 productive systems', 'Mother with less than two duties', 'No participation in events', 'Between 1 and 5 agricultural products', 'Assistant in associations', 'Between 1 and 2 chemicals', 'No technical assistance', 'No processed products', 'No fertilizers', 'Decisions 1', 'No associations', 'No accounting records' and 'Less than two livestock systems'.

This means that farms with positive coordinate tend to have efficient waste management practices, mainly used in composting; they produce and transform a large part of their raw material and have technical assistance and consultancy services for organic production. On the other hand, they have adequate spaces for their agricultural activities, possibly directed towards agroindustry and/or livestock production. Farmers are characterized for participating, training, and attending events offered by the associations they belong to, or other governmental entities, aspects observed in the adoption of practices aimed at organic production.

Component two was characterized by the following variables: children age ($R^2 = 0.4433$, $p = < 0.01$), type of work ($R^2 = 0.3606$, $p = < 0.01$), adolescent work ($R^2 = 0.2791$, $p = < 0.01$), children's education level ($R^2 = 0.3307$, $p = < 0.01$), environmental problems ($R^2 = 0.2475$, $p = < 0.01$), farm work ($R^2 = 0.2269$, $p = < 0.01$), farm money ($R^2 = 0.2635$, $p = < 0.01$), chemical inputs ($R^2 = 0.2433$, $p = < 0.01$), agricultural production ($R^2 = 0.2090$, $p = < 0.01$), farm decisions ($R^2 = 0.1685$, $p = < 0.01$), agricultural communication ($R^2 = 0.2635$, $p = < 0.01$) and father age ($R^2 = 0.2183$, $p = < 0.01$).

The coordinates of the following categories are positive: 'Children between 10.1 and 20', 'Adolescent with 3 or more duties', 'High school children', 'Parent's money', 'If there are environmental problems', 'All members', '3 or more chemicals', 'Parents' Decisions', 'Parents between 35.1 and 50 years old', 'Less than 7 productive systems', 'Between 3 and 5 agricultural waste management processes', and 'Family labor'; while the following categories are negative: 'Contracted labor', 'Adolescent with less than 2 tasks', 'No environmental problems', 'Between 1 and 2 intermediaries', 'Children without education level', 'Between 1 and 2 education systems', '11 or more productive systems', 'Non-chemical', 'Decisions 1', 'Mother with no education level', 'Parents' money', 'Less than 2 agricultural waste management processes' and 'Over 30.1 year-old children'. The positive findings in this dimension show that the decisions and money management are made jointly by father and mother, the average age of the parents ranges between 35.1 and 50 and children between 10.1 and 20, with secondary education; regarding the farm, it is evident that most of the work is carried out by the family members, they have environmental problems associated. With respect to waste management, it is evident that they manage agricultural waste, whether in composting, fertilizers, or food for livestock.

A dendogram is a diagram that shows the hierarchical relationship between objects. It is most commonly created as an output from hierarchical clustering. Cluster analysis (CA) (Figs 3 and 4) classified three farm groups. The first group was called '*Organic Producers in Transition*' (*OPT-1*), which consists of 27 farms (38.03%), the second, '*Conventional Producers in Transition Process to Organic Production*' (*CPTPOP-2*), which comprises 15 farms (21%), and finally, the third one, '*Conventional Producers Interested in Organic Production*' (*CPIOP-3*), conformed by 29 farms (40.84%); In this regard, studies conducted by Fargue -Lelièvre et al. (2011), Tuesta et al. (2014), Petit & Aubry (2015) and Cleves-Leguízamo & Jarma-Orozco (2014), have found the organic management component is discriminatory.

The most significant variables and categories in the characterization of producers ($p < 0.01$) are shown in Table 1 of supplementary material.

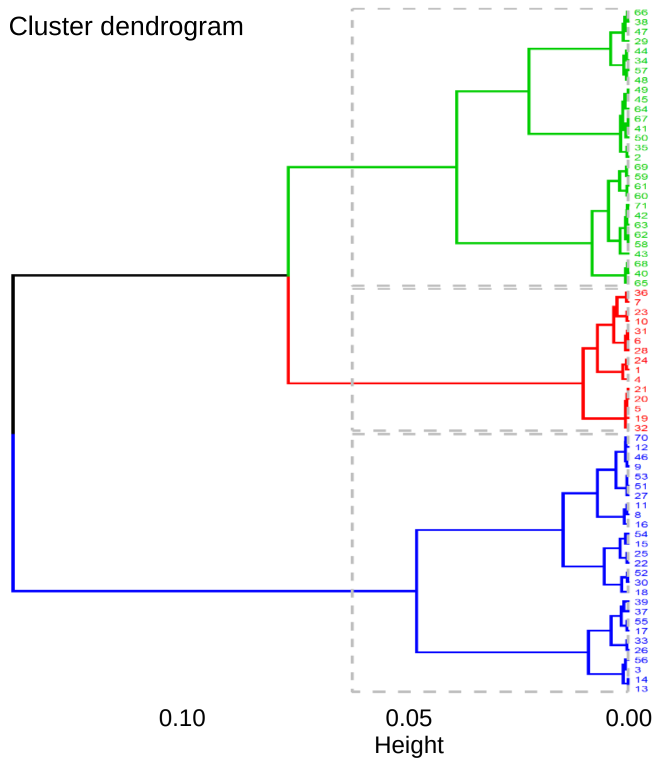


Figure 3. Grouping of production farms (*OPT-1blue*), (*CPTPOP-2red*) ((*CPIOP-3green*).

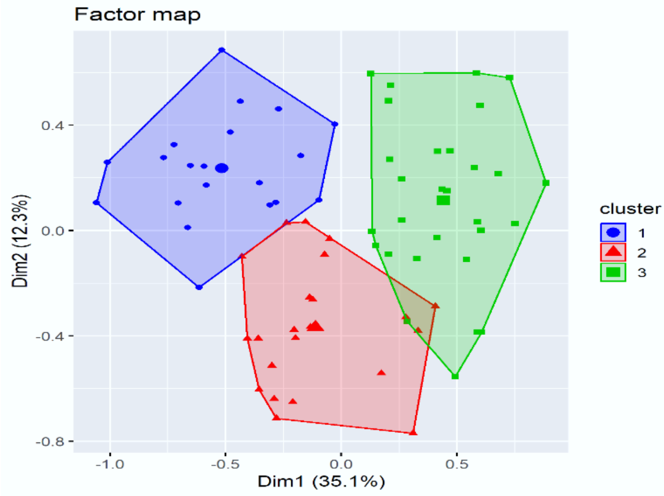


Figure 4. Grouping of producers TIF.

OPT-1, were characterized for being the most advanced organic producers due to their farms infrastructure, diversification of agricultural and livestock systems, with high production and processing of inputs. Socially, they are established nuclear families where team work prevails, and belong to associations at management level, mainly. Regarding the environment component, these producers evince the best water management and agricultural waste practices; their agroecological practices and use of organic fertilizers are extensive and many do not resort to chemical alternatives. Finally, in the technological dimension, they count on technical assistance services from one or more entities, and participate in events with access to different means of agricultural communication.

CPTPOP-2, were characterized because their infrastructure, number of agricultural production systems, elaboration and processing of inputs are lower compared to *OPT*, but higher than *CPIOP*. At a social level, the work is carried out by one or two members. Unlike the first group, a large part of these producers is associated, acting as administrative staff or associates. Their family group is not defined since most of the families do not have one or more members and decision-making and money management are carried out only by one person. Regarding the environmental aspect, this group of producers performs between 1 and 2 water management processes, some of them lack an irrigation system, agricultural waste management is scarce, resulting in a large number of environmental problems; concerning agroecological practices and use of organic fertilizers, many of them are in the implementation phase, often resorting to chemical management. Finally, at technological level, there are shortcomings in technical assistance services; however, they actively participate in events and have affordable communication systems.

CPIOP-3, were characterized by having insufficient infrastructure systems, low input processing and transformation rates, and little diversity of agricultural and livestock production systems; socially, work on the farm is carried out by one or two members, they are incipient producers in organic production, many of them do not belong to any associations and/or participate on an occasional basis. At environmental level, water and waste management is scarce, environmental problems are frequent, chemical management remains the main management alternative on the farm, and both agroecological practices and the use of organic fertilizers are just starting to be implemented. At a technological level, most of these producers do not count on any kind of technical assistance service, and most of the times they do not participate in events.

DISCUSSION

The agroecological transition is defined as the gradual change that farmers undergo to adapt and move from more conventional towards agroecological farming principles, encompassing technological, societal, institutional and organisational changes in the food system (Tittonell, 2014). Although the transition to agroecology follows general principles, each particular farm has a unique way to adopt and adapt practices and management strategies. According to Weltin et al. (2017), the challenges towards agroecological transitions are not the same for all farmers as farmers differ in objectives and values and are embedded in different social and ecological contexts. To assess the implications of farm diversity for promoting agroecological transitions, one main challenge need to be addressed, it refers to a conceptual and empirical understanding of how to assess the diversity of farmers within transition processes. Earlier studies have sought to understand the diversity of farmers through the notion of ‘farming styles’ or ‘farm typologies’, which distinguish different groups of farmers on the basis of the strategies that they pursue, as well as farm structural variables (Kansiime, 2018).

Four levels of the agroecological transition process were set by Gliessman et al. (2007) and Altieri et al. (2017), to transform conventional systems characterized by monocultures highly dependent on external inputs, into diversified systems that favor ecological services and replace, as far as possible, synthetic inputs external to the system:

Level 1: Increase in conventional practices efficiency to reduce the consumption and use of costly, scarce, or environmentally harmful inputs.

Level 2: Substitution of synthetic inputs by alternative or organic ones. The goal is to replace toxic products with more environmentally friendly ones. However, the basic structure of the agroecosystem is not strongly altered.

Level 3: Redesign of the agroecosystem so that it works on the basis of a new set of ecological processes. Thus, rather than finding healthier ways of solving problems such as pests and/or diseases, their appearance is prevented through the design of agroecosystems with diversified management and structure.

Level 4: Change of ethics and values, thinking of the two most important components of food systems, those that produce the food and those that consume the products.

To start a transition process, a series of sequential steps do not need to be completed, but because it is such a complex process, several criteria need to be considered simultaneously. This determines the need to define the productive system starting situation, and according to this scenario, proposes the strategies for the transitional process. This is why it is important to typify producers as a diagnostic tool to know their current status. In this sense, there are three key criteria of the complex reality to consider (Berkes et al., 2000): 1. The structural attributes of the particular agroecosystem; 2. The local environmental knowledge of the farmer or farming family that makes the decisions and manages the system functioning; 3. The contextual factors that condition the possibilities of developing a transition process.

In this research four dimensions (economic, social, environmental, and technological variables) studied, grouped important variables in the typology of organic producers in the Sumapaz region. In this regard, Madry et al. (2013) stated that, to classify producer systems, social, environmental, as well as economic and technical variables should be integrated in the study of typologies. At economic level, '*Infrastructure*', '*Agricultural and livestock production*', variables and '*Produced and transformed inputs*' were those that showed highly significant differences (p-value < 0.01) among the three groups. Others, such as '*Labor*', and '*Loans*' were significant (p-value < 0.05).

74.7% of cumulative variability in the eight optimal components was similar to that of Cleves-Leguízamo & Jarma-Orozco (2014), who found 77.2% in the characterization and typology of citrus systems in the department of Meta. By combining the first two components (47.4%), a similarity was demonstrated with Chatterjee et al. (2015) who obtained 47.1%, Goswami et al. (2014) 43.7%, Cortez-Arriola et al. (2015) 45.2%, and Perea et al. (2014) 49.4%; higher than that found by Martin-Collado et al. (2015) with 26.6% and Choisis et al. (2012) 27.2%, and a lower variance percentage with respect to Righi et al. (2011) 65.1%.

Infrastructure has been found as a conditioning factor for organic production (Merma & Julca, 2012). In this regard, Cortez-Arriola et al. (2015), noted, as a constraint, the low availability of specific facilities for milking; likewise, Mena et al. (2016) detected deficiencies in the lack of specific housing for newborn lambs in 68% of the farms surveyed. The diversification of production systems is another relevant aspect in the typology of organic producers (Magcale-Macandog et al., 2010; Goswami et al., 2014; Chatterjee et al., 2015; Petit & Aubry, 2015; Haileslassie et al., 2016). Regarding this, Petit et al. (2010), suggested that there should be a link between diversification, technical management, and work organization for systems of organic vegetable crops. On the other hand, Nowak et al. (2015), in a study of nutrient recycling in organic farms, confirmed the benefit of diversity in agricultural production to improve the recycling of nutrients and recommended the design of agricultural policies to promote diversity in rural territories.

Another economic factor that allowed the classification of producers in a significant way was '*workforce*' (Gafsi et al., 2010; Cleves-Leguízamo & Jarma-Orozco, 2014). 84% of the producers surveyed in this study claim to have family workforce (FWF) and the remaining 16%, hired labor. Studies conducted by Choisis et al. (2012) noted the availability of family workforce as an influencer in the differentiation of farms, contrary to Cortez-Arriola et al. (2015), who reported that the use of hired labor was a differentiating factor between surveyed producers. On the other hand, Dinis et al. (2014), noted that farms with family workforce are probably more innovative in terms of sustainability; which allows us to deduce a possible approach of producers in the Sumapaz region with FWF (84%) towards innovation, and in the medium and/or long term towards sustainability.

The social dimension was characterized through '*Farm work*', '*Farm change*', '*Mother duties*', '*Association*', '*members of an organization*', '*Children age*', '*Mother education level*', and '*Children education level*', being statistically significant (p-value < 0.01) in the typology of producers in the Sumapaz region.

Being members to one or more associations was a relevant aspect in the classification of producers in transition to organic agriculture. According to this, Cleves-Leguízamo & Jarma-Orozco (2014) found that not being part of an association, the lack of technical assistance, and the low level of education result in not having a main agroecological structure, this was corroborated in group III (CPIOP) with 51.7% respondents not being members of any association, in comparison with group I (OPT, in which 85.2% were associated; similar results were reported by Tuesta et al. (2014), which in the typology of Cacao farms in Peru, found that in two of their groups, 90% belonged to an organization. Moreover, the educational level is a differentiating factor in typologies of agricultural producers (Magcale-Macandog et al., 2010; Pienaar, 2013; Chatterjee et al., 2015).

Romero (2009) found that in hog farms of the Sumapaz region the predominant level of education of decision-makers in production systems is primary education (59.3%), similar to what this study found for parents (54.0%), and contrary to reports by Claves & Jarma (2014), who found that 83.12% of citrus producers did not complete their primary education. Regarding age, Dinis et al. (2014) noted that organic producers are in average 46 years old, with 8 years of experience in organic agriculture; on his part, Pienaar (2013) reported in his study on typology of small farmers that most of the respondents were heads of household older than 60 years old, with very little education. The foregoing is similar to what was found in this study, as 53% of men are older than 50 years old.

Regarding farm work, 96% of the respondents answered that at least one family member performs the works; comparable results were reported by Mena et al. (2016), who affirmed that 88% of the total workers, mostly men, were members of the family. On the other hand, Lima-Vidal (2013) found that rural women in the semi-arid region perform most of the technical, administrative, and managerial farm activities, which agrees with the variable '*Mother duties*', which in group I (POTr) resulted in 92.53% of farms with the mother performing three or more duties. Finally, religion was not a discriminatory variable in this study; however, Keshavarz & Karami (2010), in a drought management study with farmers in Iran, reported that 'praying' is an important variable when setting groups.

The environmental dimension was typified according to the following variables: '*Water management*', '*Agricultural waste management* (AWM)', '*Irrigation*', '*Chemical inputs*', '*Agroecological practices*', and '*Organic fertilizers*', which were statistically significant (p-value < 0.01). It was found that water is a crucial factor in the typology of agricultural producers. In this context, *irrigation* is a key variable that influences the classification of the groups (Righi et al., 2011; Merma & Julca, 2012). Haileslassie et al. (2016) showed that, on average, 25% of the farms surveyed had access to irrigation wells and that the main water supply source to these farming systems was rain. Contrary to this, this study found that 62% of the producers in the Sumapaz region have access to at least one irrigation system; however, as a water resource, 92.9% also use rain.

Regarding the *use of water*, Mena et al. (2016) noted that the availability of drinking water was a problem, especially in the summer, since 70% of the farms were not connected to the aqueduct network; in this study, 66.2% of the families count on aqueduct services; however, the effect of the current dry season was not considered. With regard to waste management, Escobar et al. (2012), showed that some producers do not perform recycling or waste management practices, and some reuse domestic water; likewise, Nyaga et al. (2015), noted that 75% of farmers use cow manure as a source of organic fertilization in corn crops; in this study, waste management practices are scarce, with groups II and III showing that 100% and 89.6%, respectively, carry out less than two waste management practices.

In regard to '*Chemical inputs*' and '*Agroecological practices*', it is observed that producers in groups II and III use chemical inputs and scarce adoption of agroecological practices. Regarding this, Tittonell et al. (2010), Fargue-Lelièvre et al. (2011), Meylan et al. (2013) and Nyaga et al. (2015) point out that the use of pesticides and fertilizers are discriminatory variables. Finally, Dinis et al. (2014) concluded that organic farming has a significant effect on sustainability practices.

Regarding '*Technological variables*', 'Participation *in events*', '*Agricultural communication*', and '*Technical Assistance*' showed statistically significant differences (p-value < 0.01). Cleves-Leguízamo & Jarma-Orozco (2014), stated that technical assistance was the biggest limitation in the classification of citrus systems, and that only 41.75% of farmers said having received some type of assistance, and Romero (2009) pointed out that 50.6% of pig farmers in the Sumapaz region reported counting on technical assistance. According to this, it was found that 56% of respondents have received some type of assistance. Regarding the adoption of organic agriculture practices, Dinis et al. (2014) highlighted the role of universities and public advisory services (technical assistance) as representatives of a potential impact of innovation policies aimed at sustainability.

Regarding agricultural communication and participation in events, in a research on the behavior of farmers towards ecological conservation, Deng et al. (2016) determined that the influence of neighbors was the most powerful behavior controller, which means that people who reside near farmers could be ambassadors of ecological achievements to positively modify farmers' behavior, and Romero (2009) showed that 70.4% of producers did not participate in technology transfer events. Accordingly, group I (POTr) showed the highest participation in events and in the media, in comparison to groups II and III.

According to the variables evaluated in our typology study, which showed three groups in different transition process to agroecological production, actions that promote sustainable productions can be generated. In this regard, in Latin America, positive studies related to agroecological transition have been reported. Flores & Sarandón (2015), studied this transition in Argentine horticultural gardens, finding that after three years of evaluation, new technologies were improved and adopted. In Costa Rica, Babin (2015) noted the agroecological practices that benefited coffee producers in the area. On a negative level, Ferreira et al. (2013) studied the changes in the production of coffee mixed with timber trees was not profitable due to the decrease in yield.

In another study, Mancini et al. (2018), report that farming systems in the Zona da Mata (Brazil) are inherently complex and diverse. Despite the aim of typology approaches to identify discrete groups, in reality farm diversity can best be understood as a continuum where different farm types can co-evolve, interact and overlap. In fact, agroecological transitions may also be understood as a process in which farmers move along an infinite continuum, and it is therefore difficult to draw a sharp line that separates agroecological from non-agroecological farmers, as well as a specific end point of transition.

To our knowledge, this is the first study that developed farm typologies in Sumapaz Region, specifically to understand and analyse a long-term process of agroecological transition, focusing on changes at farm level. We used participatory methods to interest farmers to participate in a collective process of co-creation of knowledge. This was also important to generate a collective understanding of agroecology transition process. According to Mccune & Sánchez (2018), this collective understanding is relevant to increase awareness about agroecological ideas, farms and practices, as well as to identify opportunities and barriers for promoting agroecological transitions.

Agroecology gathers several ways of thinking, with a holistic approach, in which agronomic, natural and social knowledge areas converge, reaching a synergic dimension, which from the perspective of Guzman et al. (2000), promotes the ecological management of agricultural systems through collective forms of social action that redirect co-evolution between nature and society, based on strategies from the local dimension, promoting cultural and ecosystemic diversity, as a starting point for alternative agriculture and for the establishment of dynamic and sustainable rural societies.

CONCLUSIONS

This typology study showed 3 clearly differentiated groups based on their characteristics associated with the implementation of management strategies and practices in crops, social features, environmental management, and technology transfer. Producers show variable degrees of conversion to sustainable agricultural production, which defined the discrimination in groups of organic producers in transition, conventional producers in transition to organic production, and conventional producers interested in organic production. These groups were classified according to the level of progress in practices such as production and processing of inputs, diversity in agricultural production systems, number of facilities for the improvement of production, memberships and participation in associations, the level of schooling of mothers and children, the number of duties performed by mothers, the variety of agroecological practices, management of agricultural waste, use of organic fertilizers, water management, access to technical assistance, communications, and participation in events.

The resulting typology makes it possible to foresee the need for a differentiated process in at least three categories of organization, with a view to generating an effective impact on these systems so that they become in higher level of sustainability systems. Producers need to focus their attention on processes that allow them to improve their skills to help develop the required human talent and acquire the necessary social capital in terms of integration, collaborative work, trust, political and cultural capital, so that they can easily make progress in the implementation of agroecological practices, infrastructure, natural resources management and improve their living standards.

ACKNOWLEDGEMENTS. The authors would like to thank the research department of the University of Cundinamarca for the financial support for the execution of this research.

REFERENCES

Abdi, H. & Valentin, D. 2007. Multiple correspondence analysis. *Encyclopedia of measurement and statistics* **1**(2) 651–66.

Aguirre, K.F., Calvo, M.I. L., Herrán, J.I.M. 2013. New methodological procedure for the exploratory analysis of a structured table in various sets of individuals. *Journal of Spanish Statistics* **55**(182), 305–322.

Altieri, M., Nicholls, C. & Montalba, R. 2017. Technological approaches to sustainable agriculture at a crossroads: an agroecological perspective. *Journal of Sustainability* **9**(3), 349.

Babin, N.L. 2012. *Agrarian change, agroecological transformation and the coffee crisis in Costa*. Med. Press, Costa Rica, 120 pp.

Berdegué, J. & Larraín, B. 1988. Cómo trabajan los campesinos *Journal of Sociological Research and Methods* **10**(2), 63–68 (in Spanish).

Berkes, F., Colding, J. & Folke, C. 2000. Rediscovery of traditional ecological knowledge as adaptive management. *Ecological applications* **10**(5), 1251–1262.

Blazy, J.M., Ozier-Lafontaine, H., Doré, T., Thomas, A. & Wery, J. 2009. A methodological framework that accounts for farm diversity in the prototyping of crop management systems. Application to banana-based systems in Guadeloupe. *Journal of Agricultural systems* **101**(2), 30–41.

Caporal, F.R., Paulus, G. & Castobeber, J.A. 2009. *Agroecologia: uma ciência do campo da complexidade*. Brasília MDA/SAF-Press, Brasil, 111 pp. (in Portuguese).

Carrillo, B., Moreira, V.H. & González, J. 2011. Characterization and typing of milk production systems in the central-southern zone of Chile: a multivariable analysis *Idesia* **29**(1), 71–81 (in Spanish).

Chatterjee, S., Goswami, R. & Bandopadhyay, P. 2015. Methodology of Identification and Characterization of Farming Systems in Irrigated Agriculture: Case Study in West Bengal State of India. *Journal of Agricultural Science and Technology* **17**(5), 1127–1140.

Choisis, J.P., Thévenet, C. & Gibon, A. 2012. Analyzing farming systems diversity: a case study in south-western France. *Spanish journal of agricultural research* **3**(2), 605–618.

Cleves-Leguízamo, J.A. & Jarma-Orozco, A. 2014. Characterization and typification of citrus production systems in the department of Meta. *Journal of Colombian Agronomy* **32**(1), 113–121.

Contzen, S. & Forney, J. 2017. Family farming and gendered division of labour on the move: a typology of farming-family configurations. *Agriculture and human values* **34**(1), 27–40.

Cortez-Arriola, J., Rossing, W.A., Massiotti, R.D.A., Scholberg, J.M., Groot, J.C. & Tittonell, P. 2015. Leverages for on-farm innovation from farm typologies? An illustration for family-based dairy farms in north-west Michoacán, Mexico. *Journal of Agricultural Systems* **135**, 66–76.

Daloğlu, I., Nassauer, J.I., Riolo, R.L. & Scavia, D. 2014. Development of a farmer typology of agricultural conservation behavior in the American Corn Belt. *Journal of Agriculture System.* **129**, 93–102.

Deng, J., Sun, P., Zhao, F., Han, X., Yang, G. & Feng, Y. 2016. Analysis of the ecological conservation behavior of farmers in payment for ecosystem service programs in eco-environmentally fragile areas using social psychology models. *Science of the Total Environment* **550**(2), 382–390.

Dinis, I., Ortolani, L., Bocci, R. & Brites, C. 2014. Organic agriculture and sustainable practices: towards a typology of innovative farmers. Journal of Building Organic Bridges 3(2), 745–748.

Douxchamps, M.T. Van Wijk, S., Silvestri, A.S. Moussa, C. Quiros, N.Y.B., Ndour, S. Buah, L., Somé, M., Herrero, P., Kristjanson, M., Ouedraogo, P.K., Thornton, P. Van Asten, R. Zougmoré & Rufino, M.C. 2015. Linking agricultural adaptation strategies, food security and vulnerability: evidence from West Africa Reg. *Journal of Environmet* **12**(2), 68–71.

Duru, M. & Therond, O. 2015. Designing agroecological transitions: A review. *Agronomy for Sustainable Development* **35**(4), 1237–1257.

Escobar, N., Mora, J.R & Romero, N.J. 2012. Identification of microbial populations in compost of organic residues of coffee farms in Cundinamarca. *Bol. Cient. Mus. Hist. Nat.* **16**(1), 75–88 (in Spanish).

Escobar, G. & Berdegue, J. 1990. Typification of agricultural production systems. International Network of sustainable research methodology. *Journal of Agriculture* **4**(3), 88–93 (in Spanish).

Fargue-Lelièvre, A., Moraine, M. & Coléno, F.C. 2011. Farm typology to manage sustainable blackleg resistance in oilseed rape. *Agronomy for sustainable development* **31**(4), 733–743.
Ferreira, E., Méndez, V.E., Caporal, F.R. & Faria, J.C. 2013. Agroecological transition of conilon coffee (Coffea canephora) agroforestry systems in the State of Espírito Santo, Brazil. *Agroecology and sustainable food systems* **37**(4), 405–429.
Flores, C.C. & Sarandón, S.J. 2015. Evaluation of the sustainability of an agroecological transition process in family horticultural production systems of the La Plata Party, Buenos Aires, Argentina. *Journal of the Faculty of Agronomy* **114**(2), 91–98.
Giraldo, O.F. 2008. Food security and rural livestock production: the case of the rural town of Sumapaz. *Luna Azul Journal* **27**(3), 49–59 (in Spanish).
Gafsi, M., Le Tron, S. & Mouchet, C. 2010. Organic farming is it a sustainable agriculture. Development issues for sustainable organic farming in Midi-Pyrénées Région. *In'Innovation and Sustainable Development in Agriculture and Food* **28(**2), 44–49.
García, C. & Calle, L. 1998. Methodological considerations for the typification of bovine production systems from secondary sources. *Journal of Agricultural Science and Technology* **2**(2), 6–15 (in Spanish).
Gliessman, S.R. 2007. *Agroecology: The Ecology of Sustainable Food Systems*. CRC Press, Florida, 384 pp
Guzmán, C,G., González de Molina, M. & Sevilla, G.E. 2000. *Introduction to Agroecology as sustainable rural development*. Ediciones Mundi-Press, Spain, 259 pp. (in Spanish).
Goswami, R., Chatterjee, S. & Prasad, B. 2014. Farm types and their economic characterization in complex agro-ecosystems for informed extension intervention: study from coastal West Bengal, India. *Agricultural and Food Economics* **2**(1), 1–24.
Haileslassie, A., Craufurd, P., Thiagarajah, R., Kumar, S., Whitbread, A., Rathor, A., Blummel, M., Ericsson & Kakumanu, K. 2016. Empirical evaluation of sustainability of divergent farms in the dryland farming systems of India. *Ecological Indicators* **60**(9) 710–723.
Kansiime, M.K., van Asten, P. & Sneyers, K. 2018. Farm diversity and resource use efficiency: Targeting agricultural policy interventions in East Africa farming systems. NJAS. *Wagen Journal of Life Science* **85**(10), 32–41.
Keshavarz, M. & Karami, E. 2010. Adaptation of Iranian farmers to climate variability and change. *Reg Environ Change* **14**(2), 1163–1174.
Kuivanen, K.S., Michalscheck, M., Descheemaeker, K., Adjei-Nsiah, S., Mellon-Bedi, S., Groot, J.C. & Alvarez, S. 2016. A comparison of statistical and participatory clustering of smallholder farming systems–A case study in Northern Ghana. *Journal of rural studies* **45**, 184–198.
Lima-Vidal, D. 2013. Work division in family farm production units: Feminine responsibilities typology in a semi-arid region of Brazil. *Journal of arid environments* **97**, 242–252.
Madry, M., Shananhan, W. & Rowdry., J. 2013. Identifying social, economic and technical determinants of silvopastoral practices in temperate uplands: results of a survey in the Massif central region of France. *Agricultural Systems* **69**, 119–135.
Magcale-Macandog, D.B., Ani, P.A.B. & Delgado, M.E.M. 2010. Developing an empirical typology of farmers to assist agroforestry extension: a case study in Claveria, Misamis Oriental, Philippines. *Philippine Agricultural Scientist* **93**(2), 210–223.
Mancini, H., van den Berg, L., Cardoso, I.M., Ardjan, J., Bianchi, J.A., Peña-Claros, M. & Tittonell, P. 2018. Understanding Farm Diversity to Promote Agroecological Transitions. *Sustainability* **10**, 4337–4344.
Martin-Collado, D., Byrne, T.J., Amer, P.R., Santos, B.F.S., Axford, M. & Pryce, J.E. 2015. Analyzing the heterogeneity of farmers' preferences for improvements in dairy cow traits using farmer typologies. *Journal of Dairy Science* **98**(6), 4148–4161.
Mccune, N. & Sánchez, M. 2018. Teaching the territory: Agroecological pedagogy and popular movements. *Agric. Hum*. **9**(2), 1–16.

Mena, J., Dogliotti, S., García M.C. & Rossing, M. 2016. Co-innovation of family farm systems: A systems approach to sustainable agriculture. *Agricultural Systems* **10**(4), 122–131.

Merma, I. & Julca, M.O. 2012. Tipología de productores y sostenibilidad de cultivos en Alto Urubamba. *Scientia Agropecuaria* **3**(2), 149–159.

Meylan, L., Merot, A., Gary, C. & Rapidel, B. 2013. Combining a typology and a conceptual model of cropping system to explore the diversity of relationships between ecosystem services: The case of erosion control in coffee-based agroforestry systems in Costa Rica. *Agricultural Systems* **118**, 52–64.

Nowak, B., Nesme, T., David, C. & Pellerin, S. 2015. Nutrient recycling in organic farming is related to diversity in farm types at the local level. *Agriculture, Ecosystems & Environment* **204**, 17–26.

Nyaga, J., Barrios, E., Muthuri, C., Öborn, I., Matiru, V. & Sinclair, F. 2015. Evaluating factors influencing heterogeneity in agroforestry adoption and practices within smallholder farms in Rift Valley, Kenya. *Agriculture, Ecosystems and Environment* **212**, 106–118.

Perea, J.M., Blanco, I., Barba, C., Angón, E. & García, A. 2014. Organic beef farming in Spain: typology according to livestock management and economic variables. *Scientific Journal* **24**(4), 78–89.

Petit, C. & Aubry, C. 2015. Typology of organic management styles in a cash-crop region using a multi-criteria method. *Organic Agriculture Journal* **3**, 1–15.

Petit, C., Bressoud, F. & Aubry, C. 2010. The effects of transition towards short supply chains on liveability of farming systems: initial findings and further research needs. In *IFSA Oth European symposium*, Vienna, Austria, July (pp. 4–7).

Pienaar, P.L. 2013. *Typology of smallholder farming in South Africa's former homelands: towards an appropriate classification system* (Doctoral thesis). Stellenbosch University Press, 251 pp.

Righi, E., Cittadini, E.D., Mundet, C., San Martino, L., Sanz, C. & Baltuska, N. 2011. Tipología predial del sector productor de cerezas del sur de la Patagonia argentina, Agriscientia. *Agriscientia Journal* **28**(1), 85–97 (in Spanish).

Romero, J.N.J. 2009. Socio-economic characteristics and level of technological adoption in pig production systems of the municipality of Fusagasugá, Department of Cundinamarca, Colombia. *Journal of Animal Science* **2**(1), 37–43 (in Spanish).

Shaner, W.W., Philipp, P.F. & Schmehl, W.R. 1982. *Farming systems research and development; guidelines for developing countries.* Westview Press, Boulder, USA, 414 pp.

Sraïri, M.T. & Lyoubi, R. 2003. Typology of dairy farming systems in Rabat Suburban region, Morocco. *Animal Production Journal* **52**(3), 47–58.

Tittonell, P., Muriuk, A., Shepherd, K., Mugendi, D., Kaizzi, K., Okeyo, J., Verchot, L., Coe, R. & Vanlauwe, B. 2010. The diversity of rural livelihoods and their influence on soil fertility in agricultural systems of East Africa – A typology of smallholder farms. *Agricultural Systems* **103**(2) 83–97.

Tittonell, P. 2014. Ecological intensification of agriculture-sustainable by nature. *Environment Sustainable Journal* **8**, 53–61.

Tuesta Hidalgo, O., Julca Otiniano, A., Borjas Ventura, R., Rodríguez Quispe, P. & Santistevan Méndez, M. 2014. Typology of cocoa farms in the middle sub-basin of the Huayabamba river, Huicungo district (San Martín, Peru). *Applied Ecology Journal* **13**(2), 71–78 (in Spanish).

Varela, M. 2010. *Evaluation of agroecological production systems incorporating sustainability indicators in the savanna of Bogotá*. National University of Colombia Press, Bogotá, 278 pp. (in Spanish).

Weltin, M., Zasada, I., Franke, C., Piorr, A., Raggi, M. & Viaggi, D. 2017. Analysing behavioural differences of farm households: An example of income diversification strategies based on European farm survey data. *Land Use Policy* **62**, 172–184.

Supplementary Material

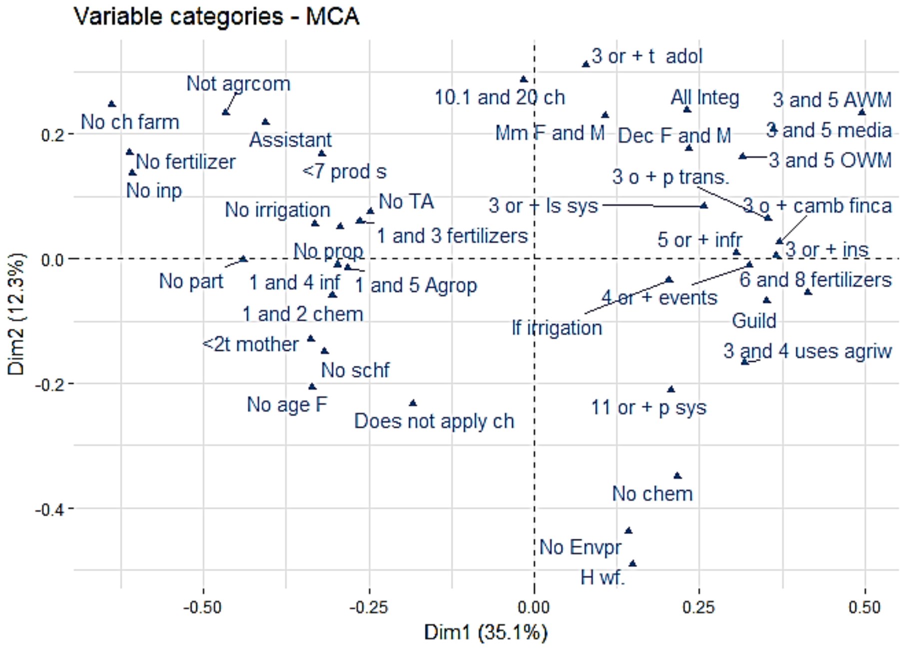


Figure 2. TIF Factor map showing the 40 main most-contributing categories to the multiple correspondence analysis (MCA). *

* Abbreviation meanings: H wf = Hired workforce; No Envpr = No environmental problems; No chem = No application of chemicals; 11 or + p sys = Eleven or more productive systems, 3 and 4 uses agriw = Three and four uses of agricultural water; Guild = Technical assistance by guild; 6 and 8 fertilizers = Between 6 and 8 organic fertilizers; If irrigation = if Irrigation system, 4 or + events = participation in 4 or more events, 5 or + infr = Five or more infrastructures, 3 or + ins = Three or more inputs produced, 3 or + farm chg = Three or more reasons for farm change, 3 or + ls sys = Three or more livestock systems, 3 and 5 OWM = Between 3 and 5 organic waste management processes, Dec F and M = Decisions made by father and mother, 3 and 5 media = Between 3 and 5 mass media, 3 and 5 AWM = Between 3 and 5 agricultural waste management processes; All Integ = Work done by all members, Mm F and M = Money management by father and mother, 3 or + t adol = Three or more tasks by adolescent, 10.1 and 20 ch = Age children between 10.1 and 20 years old, Does not apply ch = Does not apply in children, No age F = No father's age, No schf = No Level of education in father, < 2t mother = Mother with less than two tasks, No Ta = No technical assistance, 1 and 2 chem = Between 1 and 2 chemical applications, No irrigation = No irrigation system, 1 and 4 inf = Between 1 and 4 infrastructures, 1 and 5 Agrop = Between 1 and 5 agroecological practices, No part = No participation in events, 1 and 3 fertilizers = Between 1 and 3 organic fertilizers, No prop = No processed products, < 7 prod s = Less than seven productive systems, Assistant = Form of participation in association as assistant, Not agrcom = No agricultural communication, No ch farm = No changes in the farm, No fertilizer = No production of organic fertilizers, No inp = No production of inputs.

Table 1. The most significant variables and categories in the characterization of producers ($p < 0.01$)

VARIABLES	CATEGORIES	GROUPS I (n = 27)	II (n = 15)	III (n = 29)	*p*-value Chi square
ECONOMIC ASPECT					
Infrastructure	1 and 4 infrastructure	18.52%	**73.33%**	**72.41%**	**< 0.01**
	5 or more types of infrastructure	**81.48%**	26.67%	27.59%	
Agricultural production	< 7 production systems	11.11%	13.33%	**79.31%**	**< 0.01**
	Between 7 and 10 production systems	37.04%	**66.67%**	17.24%	
	11 or more agricultural production systems	**51.85%**	20.00%	3.45%	
Animal production	< 2 livestock systems	37.04%	**93.33%**	**68.97%**	**< 0.01**
	3 or more livestock systems	**62.96%**	6.67%	31.03%	
Produced inputs	No inputs	0.00%	13.33%	**41.38%**	**< 0.01**
	Between 1 and 2 inputs	25.93%	**66.67%**	**48.28%**	
	3 o more inputs	**74.07%**	20.00%	10.34%	
Processed products	No processed products	7.41%	33.33%	**58.62%**	**< 0.01**
	Between 1 and 2 processed products	**44.44%**	**60.00%**	34.48%	
	3 o more processed products	**48.15%**	6.67%	6.90%	
SOCIAL ASPECT					
Farm work	1 and 2 members	**48.15%**	**100.00%**	**68.97%**	**< 0.01**
	All members	**51.85%**	0.00%	31.03%	
Farm change	No changes farm	0.00%	0.00%	**34.48%**	**< 0.01**
	1 and 2 changes farm	**59.26%**	**86.67%**	**55.17%**	
	Three or more farm changes	**40.74%**	13.33%	10.34%	
Mother duties	< 2 mother duties	7.41%	**66.67%**	41.38%	**< 0.01**
	3 or more mother duties	**92.59%**	33.33%	**58.62%**	
Association	Associated	**85.19%**	**73.33%**	**48.28%**	**< 0.01**
	Not associated	14.81%	26.67%	**51.72%**	
Part of the organization	Administrator or associate	**66.67%**	**80.00%**	34.48%	**< 0.01**
	Assistant	11.11%	0.00%	**44.83%**	
	No part	11.11%	20.00%	13.79%	
	Operational	11.11%	0.00%	6.90%	
Children age	No age children	11.11%	**73.33%**	27.59%	**< 0.01**
	0 and 10 years	3.70%	0.00%	10.34%	
	10.1 and 20 years	**29.63%**	0.00%	**44.83%**	
	20.1 and 30 years	**37.04%**	**6.67%**	13.79%	
	Older than 30 years	18.52%	**20.00%**	3.45%	
Mother education level	No education	11.11%	**60.00%**	17.24%	**< 0.01**
	Primary school	**29.63%**	13.33%	**48.28%**	
	High school	**33.33%**	13.33%	**31.03%**	
	Higher education	25.93%	13.33%	3.45%	
Children education level	No education children	22.22%	**73.33%**	**24.14%**	**< 0.01**
	Primary school	0.00%	6.67%	17.24%	
	High school	**33.33%**	0.00%	**51.72%**	
	Higher education	**44.44%**	**20.00%**	6.90%	

Table 1 (continued)

ENVIRONMENTAL					
Water management	No water management	3.70%	0.00%	20.69%	**< 0.01**
	1 and 2 water management	**55.56%**	**93.33%**	**72.41%**	
	All water management	**40.74%**	6.67%	6.90%	
Agricultural waste management	< 2 waste management processes	**51.85%**	**100.00%**	**89.66%**	**< 0.01**
	3 and 5 waste management processes	**48.15%**	0.00%	10.34%	
Irrigation	Irrigation	**92.59%**	**60.00%**	34.48%	**< 0.01**
	No Irrigation	7.41%	**40.00%**	**65.52%**	
Chemical inputs	1 and 2 chemical inputs	22.22%	**53.33%**	48.28%	**< 0.01**
	3 o + chemical inputs	11.11%	13.33%	**51.72%**	
	Not chemicals	**66.67%**	33.33%	0.00%	
Agroecological practices	1 and 5 practices	14.81%	**53.33%**	**58.62%**	**< 0.01**
	6 and 10 practices	**66.67%**	33.33%	31.03%	
	11 and practices	18.52%	13.33%	10.34%	
Organic fertilizers	No organic fertilizers	0.00%	0.00%	**24.14%**	**< 0.01**
	1 and 3 organic fertilizers	11.11%	**33.33%**	**58.62%**	
	4 and 5 organic fertilizers	**48.15%**	**40.00%**	17.24%	
	6 and 8 organic fertilizers	**40.74%**	26.67%	0.00%	
TECHNOLOGICAL					
Participation in events	No participation	7.41%	26.67%	**34.48%**	**< 0.01**
	1 and 3 events	29.63%	33.33%	**55.17%**	
	4 o more events	**62.96%**	**40.00%**	10.34%	
Agricultural communication	No communication	7.41%	0.00%	17.24%	**< 0.01**
	1 and 2 media	**40.74%**	**100.00%**	**68.97%**	
	3 and 5 media	**51.85%**	0.00%	13.79%	
Technical assistance	No technical assistance	22.22%	**33.33%**	**68.97%**	**< 0.01**
	State	18.52%	13.33%	6.90%	
	Particular	**22.22%**	**33.33%**	24.14%	
	Guild	**37.04%**	20.00%	0.00%	

Agronomy Research **17**(6), 2260–2268, 2019
https://doi.org/10.15159/AR.19.201

Technological features of production of lactate-containing additives from milk whey fermented with lactic acid bacteria

V. V. Eveleva[1,*], T. M. Cherpalova[1], E. A. Shipovskaya[1] and N. A. Korshunova[2]

[1]All-Russia Research Institute of Food Additives – Branch of the Federal Budget Research Institution 'V.M. Gorbatov Federal Research Center for Food Systems' of RAS, 55 Liteyny ave., RU191014 Saint Petersburg, Russia
[2]Saint Petersburg National Research University of Information Technology, Mechanics and Optics (ITMO University), 9 Lomonosov str., RU191002 Saint Petersburg, Russia
[*]Correspondence: v.eveleva@yandex.ru

Abstract. Milk whey becomes a product of interest to researchers and manufacturers due to stricter environmental protection requirements. This paper discusses bioconversion of whey lactose into lactate-containing additives using microorganisms of *Lactobacillus* genus. The biotransformation of lactose from curd whey and standard solutions of cheese whey into lactic acid derivatives was assessed by the following parameters: the productivity of lactic acid bacteria, the rate of lactose fermentation, the total amount of calcium lactate and its formation rate. Selection of the medium preparation and lactic acid biosynthesis parameters based on these measurements proved to yield optimal results. Lactic acid bacteria from the subgroup of thermophilic bacilli *L. acidophilus*, *L. bulgaricus*, *L. casei*, *L. lactis*, *L. helveticus*, *L. plantarum* were also tested. The optimal synthetic activity in the terms of calcium lactate turnover and formation rate was demonstrated by *L. acidophilus* in a medium based on the concentrated whey with 8% lactose.

Key words: lactate-containing additives, lactic acid bacteria, lactose, whey.

INTRODUCTION

Milk whey processing attracts attention of researchers and manufacturers because of stricter environmental protection requirements and a concept of whey by-products valorization.

According to Mollea et al. (2013) up to 70% of the produced whey is subjected to industrial processing to obtain food and feed products. Other researchers suggest that 3.2 million tons (1.7%) of 187 million tons of curd whey produced worldwide were processed by various industries into added value products such as whey powder, whey protein isolate, whey protein concentrate (Chavan et al., 2015). Increasing the efficiency of manufacturing of products with added value from whey and reducing the unused dairy material's impact on the environment is possible with increasing the recycling capacity and introducing waste-free technologies. Russia, along with other countries, has considerable potential for producing and marketing similar added value products. Whey has unrivaled potential in this regard due to its polymorphism and exceptional properties of the principal carbohydrate. Ingredients obtained by whey bioconversion may be introduced into many products, including functional and sports nutrition and baby food (Sinel'nikov et al., 2007; Hramtsov, 2011; Nath et al., 2016).

A large number of scientific and technical developments using dairy raw material containing lactose have been produced these days. Based on recent research and patent data, the biotechnological method is one of the promising directions for whey processing (Magalhães et al., 2011; Mikhneva et al., 2013; Ryabtseva et al., 2014; Gavrilov et al., 2015; Rosolen et al., 2015). This is due to the presence of nutrients necessary to grow industrial microorganisms producing a variety of useful metabolites such as lactic acid, ethanol, and acetic acid. Microorganisms for whey processing are representatives of aerobic and anaerobic cultures.

Previously, production of butanol from cheese and curd whey by *Clostridium acetobutylicum* provided productivity of 2.66 g L^{-1}, a final concentration of 4.9 g L^{-1}, a yield of 0.3 g g^{-1}, and a selectivity of 82% w⁄w (Gonzalez–Siso, 1996; Raganati et al., 2013). Cheese whey was effectively used as a substrate for kefir-like beverages production with a composition similar to traditional milk kefir: 7.8–8.3 g L^{-1} ethanol, 5.0 g L^{-1} lactic acid, and 0.7 g L^{-1} acetic acid (Magalhães et al., 2011; Rosolen et al., 2015). Most often, lactic acid bacteria *Lactobacillus* are chosen as a producer culture. Using pure cultures of lactobacilli *L. acidophilus* and *L. casei*, whey can be transformed into products with improved dietary and functional properties and prolonged shelf life (Mikhneva et al., 2013). Additionally, lactic acid bacteria *L. jugurti* can be used to obtain calcium lactate from whey. Of interest are the data that lactobacilli are able to synthesize, along with lactic acid, various antimicrobial substances which have an inhibitory effect on the growth of microorganisms that cause food products spoilage during storage and⁄or diseases of humans and animals when consumed. This antimicrobial property is particularly valuable for manufacturers of probiotic additives and products (Raskoshnaya et al., 2016).

The metabolites with antimicrobial action derived from whey media can be incorporated into high concentration *Lactobacillus casei* biomass for fodder (Bernardez et al., 2008). In the complex treatment of patients with gastroenterological diseases, a drink based on cheese whey showed effects equal to whole milk dairy products with recognized probiotic action during clinical trials (Irkitova & Vechernina, 2010).

Processing of whey in chemical and biochemical synthesis is known to provide various derivatives. The range of the derivatives is quite wide, with more than 50 products having prospects in various fields (Sinel'nikov et al., 2007). These can be divided into lactose hydrolysates (galactose, tagatose, fucose, lactobionic acid, lactitol, galactooligosaccharides, lactosaccharose, lactosyl urea) and lactose biotransformation products (lactic acid, citric acid, ethanol, acetic acid, propionic acid, bacteriocins, antibiotics, yeast, vitamins, enzymes, proteins, and lipids).

Recent research has shown an increase in the productivity of lactic acid bacteria and subsequent intensification of whey lactic acid fermentation. For instance, Kumar et al. (2014) described a biocatalytic effect of a composite of porous de-lignified cellulose, Ca-alginate and polylactic acid on the biosynthetic activity of *L. bulgaricus* bacteria immobilized on this carrier. In experimental fermentations of cheese whey with *L. acidophilus* it was found that the average duration of the lactose biotransformation process is 48 h with the mass concentration of sodium chloride from 0.1 up to 0.8%, the

percentage of the inoculation material from 3 up to approximately 10% $^{V}\!/_{V}$, and the lactose mass fraction from 3.8 up to 4.2% (Eveleva et al., 2018).
Castro et al. (2013) showed that the growth of *L. acidophilus* and the biosynthesis of organic acids, lactic acid in particular, depended on the content of lactose in whey.

The main purposes of whey conversion, apart from the benefits for consumers, are commercial benefits for entrepreneurs and environmental protection (Shchetinin & Dorokhova, 2013). Better profit margins and environmental safety can be achieved if whey is concentrated by removing moisture, which helps to prolong its shelf life and reduce production costs. The traditional method of concentration is evaporation, in which moisture is removed by boiling the liquid at low pressure and constant temperature (Kretov et al., 2010). Considering the energy costs, the preferred method is freezing-out, due to the preservation of heat-sensitive whey components and their biological value. Considering the amino acid composition of curd whey and comparing two techniques, concentrating by freezing-out preserves more whey amino acids (Kretov et al., 2010). Lactose-containing raw materials are a source for additional produce and improved efficiency of dairy production facilities.

The purpose of this study was to identify technological features of production of lactate-containing additives from milk whey fermented with lactic acid bacteria.

MATERIALS AND METHODS

Materials used in the study

- solutions of whey subjected to experimental fermentation with lactic acid bacteria (hereinafter ‘fermented whey’);
- curd whey with lactose mass fraction from 3.8% to 4.2% produced by Losevo dairy factory LLC;
- standardized solutions of cheese whey, obtained by adding edible salt to whey in amount of 0.1% to 2.0%;
- individual strains from the subgroup of thermophilic bacteria of *Lactobacillus* genus: L. *acidophilus*, L. *bulgaricus*, L. *helveticus*, L. *lactis*, L. *plantarum*;
- organic and inorganic growth stimulants, viz.: non-granulated malt sprouts produced by Baltic Malting Company llc; the extract of such sprouts; the enzyme Celloviridin g20h with cellulase activity of 200 units g^{-1} (hereinafter ‘celloviridin’) obtained from Sibbiofarm; disodium phosphate;
- suspension of chalk in various media: drinking water, curd whey, standardized solutions of cheese whey, solutions obtained by lactic acid fermentation of whey.

Methods of processing and determination

The studies were carried out in the laboratory of the All-Russia Research Institute of Food Additives using physical, chemical and microbiological test methods.

The process of biotransformation of curd whey and cheese whey lactose into lactic acid derivatives was studied with regard to productivity (biosynthetic activity) of lactic acid bacteria, the rate of lactose fermentation, the total amount and the rate of calcium lactate formation.

The controlled parameters included the following: specific gravity, active and titratable acidity, mass fraction of lactose and calcium lactate in the solutions.

The controlled parameters were determined by the common industrial and research methods. Specific gravity was determined with hydrometry and pycnometry; active acidity was determined with potentiometry; titratable acidity was measured with acidic-basic titration; the mass fraction of calcium lactate was measured with trilonometric titration; the mass fraction of lactose was measured with Bertrand's permanganate method.

Taking the assumption that the efficiency of lactose biotransformation by lactic acid bacteria depends on the acidification activity of a microorganism, as well as the culture age, the amount of the inoculation material, method of material preparation to fermentation, the presence of stimulants in the medium, and the neutralizing agent, the experiments were planned so that all these parameters underwent variations.

All measurements were taken in triplicate, and error margins were calculated via analysis of variance to prove statistical significance with $p < 0.05$.

Experimental bioprocessing of whey using lactic acid bacteria comprised the preparation of dairy raw materials and lactic acid bacteria cultures, whey fermentation and product recovery. For whey preparation, fat phase removal, separatory sedimentation of solid particles, whey pasteurization, and addition of organic and inorganic growth stimulants was carried out.

Preparation of lactic acid bacterial culture included culture inoculation from ampoules to tubes containing sterile skim milk; keeping them in a thermostat at a temperature of 40 to 42 °C for 6 to 24 h; re-inoculation to skim milk; pasteurization at 70 °C for 10 minutes and cooling to the cultivation temperature according to the common methods of microbiology. The last generation of lactic acid bacteria was inoculated to whey pasteurized under similar conditions.

Batch fermentations of the curd whey and the standardized solutions of cheese whey were carried out in a thermostat at the temperature of (40 ± 3) °C in 0.5 and 1.0 dm^3 Erlenmeyer flasks for optimal parameters of the preparation of the nutrient medium, biosynthesis and acid neutralization.

RESULTS AND DISCUSSION

Comparative studies of the growth and production of lactic acid by the following strains of lactobacilli: *L. acidophilus*, *L. bulgaricus*, *L. lactis*, *L. helveticus* and *L. plantarum* were carried out. Among these bacteria, the highest values of productivity (up to 0.60 $g\ dm^{-3}\ h^{-1}$) and maximum acidity (up to 370 °T) were registered for *L. acidophilus*. Additionally, *L. acidophilus* had the highest resistance to the presence of salt in the environment. When fermenting standardized solutions of cheese whey with a 2% mass fraction of salt, the growth of acidophilic bacilli was maintained, though there was a decrease in biosynthetic activity.

Having selected *L. acidophilus* for further experiments, the influence of the amount and the age of the inoculation material on the growth of *L. acidophilus* was studied. It was found that the greatest efficiency of the lactose fermentation process was achieved by introducing the inoculation material into the whey at the age of 8 h and a volume fraction of 5%, as evidenced by the changes in the mass fraction of calcium lactate in the fermented solutions (Tables 1, 2).

Table 1. Mass fraction of calcium lactate in fermentation media based on curd whey at variation of the age of the inoculation material of *L. acidophilus* ($p < 0.05$)

Age of the culture, h	Mass fraction of calcium lactate, % at the duration of the fermentation process, h			
	0	24	48	72
4	0.84 ± 0.01	1.78 ± 0.01	3.35 ± 0.03	4.30 ± 0.04
6	1.0 ± 0.01	2.20 ± 0.02	3.35 ± 0.03	4.42 ± 0.04
8	0.84 ± 0.01	1.89 ± 0.02	3.65 ± 0.04	4.61 ± 0.05
12	0.94 ± 0.01	1.78 ± 0.02	3.04 ± 0.03	3.77 ± 0.03
24	0.94 ± 0.01	1.78 ± 0.02	2.72 ± 0.03	3.56 ± 0.03

Some processes involving fermentation of carbohydrates by lactic acid bacteria include the addition of organic growth stimulants to the medium. Thus, the influence of such stimulants as non-sterile and pasteurized malt sprouts, non-sterile malt sprouts with an addition of celloviridin, pasteurized aqueous extract of the malt sprouts on curd whey lactose biotransformation was further studied. Table 3 summarizes the parameters of curd whey fermentation by *L. acidophilus* with different organic growth stimulants. The data show that introducing the tested organic growth stimulants into the whey improved the efficiency of the whey fermentation process, which is reflected in an increase in the mass fraction of calcium lactate and a decrease in the mass fraction of lactose after 72 h of whey fermentation by *L. acidophilus*. However, the use of organic stimulants for this purpose in industry cannot be recommended, since large amounts of processed whey form a significant amount of deposit after fermentation, which in turn requires recycling. It is also worth noting that the introduction of non-sterile malt sprouts into the nutrient medium can produce undesirable contaminations.

Table 2. Mass fraction of calcium lactate in fermentation media based on standardized solutions of cheese whey at variation of volume fraction of *L. acidophilus* inoculation material ($p < 0.05$)

Volume fraction of the inoculant, %	Mass fraction of calcium lactate, % at the duration of the fermentation process, h		
	24	48	54
5.0	1.97 ± 0.02	3.44 ± 0.03	3.93 ± 0.03
7.5	1.84 ± 0.02	3.45 ± 0.03	3.83 ± 0.03
10.0	1.82 ± 0.02	3.35 ± 0.03	3.84 ± 0.03
12.5	1.76 ± 0.01	3.29 ± 0.03	3.67 ± 0.03

Table 3. Curd whey fermentation parameters using *L. acidophilus* with different growth stimulants ($p < 0.05$)

Growth stimulant	Percentage during fermentation	
	start of process	after 72 h
CALCIUM LACTATE		
Control (no stimulant)	1.15 ± 0.01	3.56 ± 0.04
Non-sterile malt sprouts (10% $^{w}/_{w}$)	1.05 ± 0.01	5.24 ± 0.05
Malt sprouts, pasteurized (10% $^{w}/_{w}$)	1.05 ± 0.01	5.24 ± 0.05
Non-sterile malt sprouts (2% $^{w}/_{w}$)	1.05 ± 0.01	4.70 ± 0.04
Non-sterile malt sprouts (2%) with celloviridin (0.25% $^{w}/_{w}$)	1.05 ± 0.01	4.70 ± 0.04
Water extract from malt sprouts, pasteurized (1 : 10 ratio)	1.05 ± 0.01	4.61 ± 0.04

Table 3 (continued)

LACTOSE		
Control (no stimulant)	4.94 ± 0.05	1.30 ± 0.02
Non-sterile malt sprouts (10% $^{w}/_{w}$)	4.78 ± 0.04	0.29 ± 0.01
Malt sprouts, pasteurized (10% $^{w}/_{w}$)	4.78 ± 0.04	0.29 ± 0.01
Non-sterile malt sprouts (2% $^{w}/_{w}$)	4.78 ± 0.04	0.92 ± 0.01
Non-sterile malt sprouts (2%) with celloviridin (0.25% $^{w}/_{w}$)	4.75 ± 0.04	0.79 ± 0.01
Water extract from malt sprouts, pasteurized (1 : 10 ratio)	4.78 ± 0.04	0.55 ± 0.01

Inorganic growth stimulants such as disodium phosphate are used in milk protein production as an effective and safe source of inorganic nutrients for the bacteria *L. acidophilus*. It was found that disodium phosphate stimulates the biosynthetic activity of the microorganism and contributes to a significant increase in productivity of *L. acidophilus* during whey fermentation. The data in Table 4 confirm that the introduction of disodium phosphate in the nutrient medium in the amount of 2% helps to achieve the highest biosynthetic activity of *L. acidophilus* and provides optimal pH of the medium for the development of the bacteria: 5.2 to 5.6.The effect of various neutralizing agents (calcium carbonate, sodium carbonate, potassium carbonate and aqueous ammonia solution) on the fermentation rate of whey lactose by *L. acidophilus* was assessed. The addition of powdered calcium carbonate, sodium carbonate and potassium carbonate did not maintain the pH of the fermented solutions within an acceptably narrow range. The use of calcium carbonate in the form of an aqueous suspension is impractical due to dilution of fermented solutions and sedimentation instability of the suspension. Neutralizing the fermented whey with a 25% ammonia solution provided the highest rate of lactose fermentation (0.53 ± 0.01 g h^{-1}) among the neutralizing agents tested. Even though this value is still not up to the standard (Hramtsov, 2011), a 25% ammonia solution appeared to be the best agent from the list above.

Table 4. Productivity of *L. acidophilus* during fermentation of cheese whey with different concentrations of disodium phosphate in fermentation media ($p < 0.05$)

Mass fraction of disodium phosphate, %	Productivity, g dm^{-3} h^{-1}
0	0.65 ± 0.04
0.5	0.80 ± 0.02
1.0	0.86 ± 0.02
2.0	0.90 ± 0.03
3.0	0.81 ± 0.01

Taking into account the findings by Kumar et al. (2014) that the growth and the biosynthetic activity of *L. acidophilus* depends on the content of lactose in whey, experimental fermentation of curd whey with the mass fraction of lactose from 4% to 10% was done. The rate of formation and and the total amount of calcium lactate was found to correlate with the mass fraction of lactose and the duration of the biosynthesis. The greatest biosynthetic activity was achieved on a whey medium with 8% lactose (Figs 1, 2).

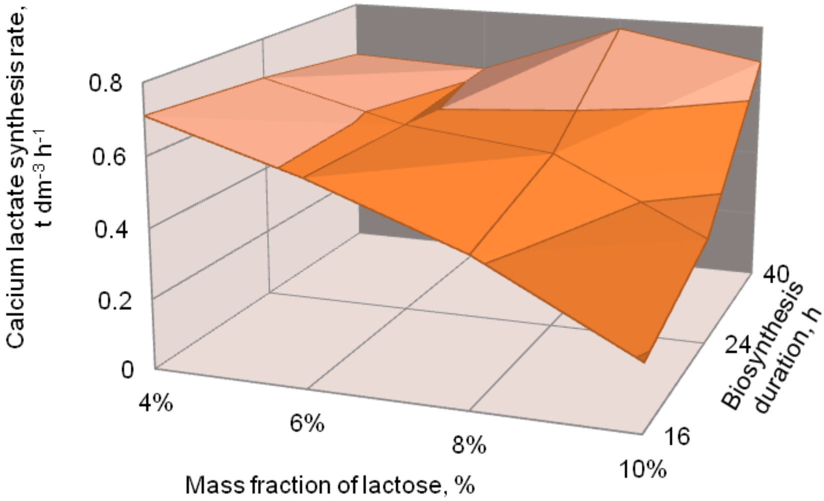


Figure 1. Response surface of the rate of calcium lactate formation to lactose mass percentage in whey media and the duration of biosynthesis.

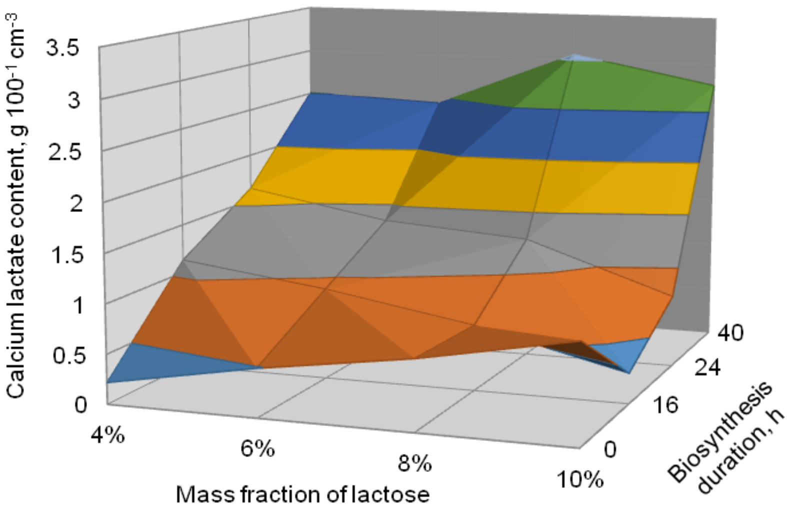


Figure 2. Response surface of the content of calcium lactate to lactose mass percentage in whey media and the duration of biosynthesis.

The observed correlations suggest that in order to improve the efficiency of whey processing, production lines of lactate-containing food and feed additives could benefit from a material concentration step.

CONCLUSIONS

It was established that the greatest efficiency of lactose fermentation can be reached by *L. acidophilus* with introducing the inoculation material into whey at the age of 8 h and a volume fraction of 5%.

It was demonstrated that introducing organic growth stimulants (non-sterile and pasteurized malt sprouts, non-sterile malt sprouts with an addition of celloviridin, pasteurized aqueous extract of the malt sprouts) into whey contributes by increase in the mass fraction of calcium lactate and decrease in the mass fraction of lactose after 72 h of whey fermentation. However, the industrial use of organic stimulants cannot be recommended due to significant amounts of sediments formed.

Regarding inorganic growth stimulants, disodium phosphate proved to stimulate the biosynthetic activity of the bacteria *L. acidophilus* and to contribute to a substantial increase in their productivity during whey fermentation. The introduction of disodium phosphate in the nutrient medium in the amount of 2% helps to achieve the highest biosynthetic activity of the bacteria *L. acidophilus* and provides optimal pH of the medium for the development of *L. acidophilus* (5.2 to 5.6).

Having tested the effect of various neutralizing agents (calcium carbonate, sodium carbonate, potassium carbonate and aqueous ammonia solution) on the rate of fermentation of whey lactose by *L. acidophilus*, it was found that calcium carbonate was the agent of choice from the list above.

Correlations between the formation rate and the total amount of calcium lactate, on one end, and the mass fraction of lactose and the duration of biosynthesis, on the other, were found. In this regard, the greatest biosynthetic activity of the microorganism can be achieved on a medium based on concentrated whey containing 8% lactose.

REFERENCES

Bernardez, P.F., Amado, I.R., Castro, L.P. & Guerra, N.P. 2008. Production of a potentially probiotic culture of *Lactobacillus casei subsp. casei* CECT 4043 in whey. *International Dairy Journal* **18**(10–11), 1057–1065. doi: https://doi.org/10.1016/j.idairyj.2008.05.004

Castro, W.F., Cruz, A.G., Rodrigues, D., Ghiselli, G., Oliveira, C.A.F., Faria, J.A.F. & Godoy, H.T. 2013. Effects of different whey concentrations on physicochemical characteristics and viable counts of starter bacteria in dairy beverage supplemented with probiotics. *Journal of Dairy Science* **96**(1), 96–100. doi: https://doi.org/10.3168/jds.2012-5576

Chavan, R.S., Shraddha, R.C., Kumar, A. & Nalawade, T. 2015. Whey based beverage: its functionality, formulations, health benefits and applications. *Journal of Food Processing and Technology* **6**(10), 495. doi: https://doi.org/10.4172/2157-7110.1000495

Eveleva, V.V., Cherpalova, T.M. & Shipovskaya, A.A. 2018. Study of biotransformation of whey into lactate-containing products. *Dairy Industry* **7**, 40–42. doi: https://doi.org/10.31515/1019-8946-2018-7-40-42 (in Russian).

Gavrilov, G.B., Prosekov, A.Y., Kravchenko, E.F. & Gavrilov, B.G. 2015. *Whey processing handbook. Technologies, processes and devices, membrane equipment.* Professiya, Saint Petersburg, 176 pp. (in Russian).

Gonzalez-Siso, M.I. 1996. The biotechnological utilization of cheese whey: A review. *Bioresource Technology* **57**(1), 1–11. doi: https://doi.org/10.1016/0960-8524(96)00036-3

Hramtsov, A.G. 2011. *The phenomenon of whey.* Professiya, Saint Petersburg, 804 pp. (in Russian).
Irkitova, A.N. & Vechernina, N.A. 2010. Biotechnology of a probiotic drink based on milk (cheese) whey. *Altai State University Newsletter* **3⁄1**(67), 30–32 (in Russian).
Kretov, I.T., Ovsyannikov, V.Y. & Panchenko, S.L. 2010. Effect of concentration on amino acid composition of whey. *Food Industry* **1**, 44–45 (in Russian).
Kumar, M.N., Giallelia, A., Masson, J.B., Giallelia, A.-I., Kandylis, P., Bekatorou, A., Koutinas, A.A. & Kanellakia, M. 2014. Lactic acid fermentation by cells immobilised on various porous cellulosic materials and their alginate⁄polylactic acid composites. *Bioresource Technology* **165**, 332–335. doi: https://doi.org/10.1016/j.biortech.2014.02.110
Magalhães, K.T., Dragone, G., Pereira, G.V. de Melo, Oliveira, J.M., Domingues, L., Teixeira, J.A., Silva, J.B.A. & Schwan, R.F. 2011. Comparative study of the biochemical changes and volatile compound formations during the production of novel whey-based kefir beverages and traditional milk kefir. *Food Chemistry* **126**(1), 249–253. doi: https://doi.org/10.1016/j.foodchem.2010.11.012
Mikhneva, V.A., Kulikova, I.K., Yevdokimov, I.A. & Volodin, D.N. 2013. Method of the production of whey-based product. Patent RU2493718 C1 (in Russian).
Mollea, C., Marmo, L. & Bosco, F. 2013. Valorisation of cheese whey, a by-product from the dairy industry. *Food Industry*: Ed. by Innocenzo Muzzalupo, 4879. doi: https://doi.org/10.5772/53159.
Nath, A., Verasztó, B., Basak, S., Koris, A., Kovács, Z. & Vatai, G. 2016. Synthesis of lactose-derived nutraceuticals from dairy waste whey: a review. *Food and Bioprocess Technology* **9**(1), 16–48. doi: https://doi.org/10.1007/s11947-015-1572-2
Raganati, F., Olivieri, G., Procentese, A., Russo, M.E., Salatino, P. & Marzocchella, A. 2013. Butanol production by bioconversion of cheese whey in a continuous packed bed reactor. *Bioresource Technology* **138**, 259–265. doi: https://doi.org/10.1016/j.biortech.2013.03.180
Raskoshnaya, T.A., Semenikhina, V.F., Rozhkova, I.V. & Begunova, A.V. 2016. Development of culture medium and modes of cultivation of *Lactobacillus reuteri* for synthesis of bacterial concentrate. *Equipment and Technology of Food Production Facilities* **42**(3), 56–62 (in Russian).
Rosolen, M.D., Gennari, A., Volpato, G. & Volken de Souza, C.F. 2015. Lactose hydrolysis in milk and dairy whey using microbial β-galactosidases. *Enzyme Research* **7**. doi: https://doi.org/10.1155/2015/806240
Ryabceva, S.A. 2014. *Microbiology of secondary dairy raw materials.* North Caucasian Federal Univ. Publ., Stavropol, 174 pp. (in Russian).
Shchetinin, M.P. & Dorokhova, A.S. 2013. Production and processing of whey in Russia and Altai region. *Polzunovsky Vestnik* **4–4**, 80–84 (in Russian).
Sinel'nikov, B.M., Hramtsov, A.G., Yevdokimov, I.A., Ryabtseva, S.A. & Serov, A.V. 2007. *Lactose and its derivatives.* Professiya, Saint Petersburg, 768 pp. (in Russian).

Agronomy Research **17**(6), 2269–2276, 2019
https://doi.org/10.15159/AR.19.222

Physiological disorders affect apple susceptibility to *Penicillium expansum* infection and increase probability for mycotoxin patulin occurrence in apple juice

L. Heinmaa[1,*], P. Põldma[1], K. Loit[2], R. Kiiker[2] and U. Moor[1]

[1]Estonian University of Life Sciences, Institute of Agricultural and Environmental Sciences, Chair of Horticulture, Kreutzwaldi 1, EE51006 Tartu, Estonia
[2]Estonian University of Life Sciences, Institute of Agricultural and Environmental Sciences, Chair of Plant Health, Kreutzwaldi 1, EE51006 Tartu, Estonia
[*]Correspondence: lagle.heinmaa@student.emu.ee

Abstract. *Penicillium expansum* infection of apples and mycotoxin patulin (PAT) production has previously been associated with many pre- and postharvest factors other than physiological disorders. In the current study, 'Antei' and 'Krameri tuviõun' apples with and without bitter pit (BP) symptoms and 'Talvenauding' apples with and without superficial scald (SS) symptoms were used in order to determine if the named physiological disorders may influence susceptibility to *P. expansum* infection and PAT production. Apples were inoculated with 10 µL *P. expansum* spore suspension with the concentration of 1×105 conidia mL^{-1} and stored at 24 °C with relative humidity (RH) 80%. After 7 and 11 days, lesion diameters were measured, and apples were pressed into juice. PAT content was determined in pasteurized juice. Two cultivars out of three showed that in fruit with physiological disorders, *Penicilllium* infection and PAT production proceeded significantly faster compared to apples, which did not have physiological disorders. SS increased the risk for PAT occurrence in juice more than BP: while the juice pressed from BP–affected apples with no visual signs of fungal diseases did not contain PAT, juice pressed from apples with SS contained PAT three times above legislative limits defined by the World Health Organization (50 µg L^{-1}).

Key words: bitter pit, blue mould, lesion diameter, superficial scald.

INTRODUCTION

Physiological disorders like bitter pit (BP) and superficial scald (SS) have commonly been described affecting visual quality of apple fruit and therefore decreasing their market value. Less attention has been paid to study whether physiological disorders can be possible infection points for mycotoxin-producing fungi, especially *Penicillium expansum*, the major producer of patulin (PAT) (Morales et al., 2007). SS is caused by the accumulation of oxidative breakdown products of α-farnesene, which results in the death of the hypodermal and epidermal cells (McGlasson, 1996). The disorder is characterized by an uneven browning or bronzing of the skin along with the development of skin wrinkling and pitting with increasing severity (Rupasinghe et al., 2000; Savran & Koyuncu, 2016). The cause of BP has been associated with calcium (Ca) deficiency:

Ca sprays and Ca applications to soil during growing season or Ca solution dips at postharvest are widespread practices to supply Ca and decrease BP in apples (Ferguson & Watkins, 1989; Fallahi et al., 1997; Blanco et al., 2010; Torres et al., 2017). BP is a physiological disorder that is defined as brown, corky and roundish lesions, which can develop in apples before and after harvest (Jarolmasjed et al., 2016). In Estonia, apples 'Krameri tuviõun' and 'Antei' are susceptible to BP among commercially grown cultivars. In other countries, several apple cultivars are susceptible to BP like 'Sinap Orlovskij', 'Ligol', 'Free Redstar', 'Rajka', 'Topaz', 'Sampion', 'Honeycrisp', 'Golden Delicious' and 'Granny Smith' (Lanauskas & Kviklienė, 2006; Bryk & Broniarek-Niemiec, 2008; Valiuškaitė et al., 2009; Lanauskas et al., 2012; Zúñiga et al., 2017).

Earlier experiments in Estonia have shown that BP in 'Krameri Tuviõun' was not reduced by Ca treatment; instead it correlated negatively with Mg and P content and Mg:Ca ratio in apples (Moor et al., 2006). It appears that BP is a complex disorder, which is not easy to eliminate. Saure (2002) stated that BP is essentially the result of a gibberellin-induced increased susceptibility of the cell membranes to stress, and Ca only reduces the effect of gibberellins.

Besides reducing the market value of apples, BP can also be responsible for apple susceptibility to different storage diseases. For instance, an earlier study by Holb et al. (2012) showed that pre-harvest Ca sprays resulted in significant brown rot reduction on apple fruit over a 6-month storage period. So far the named disorders have not been associated with susceptibility to patulin-producing fungi and therefore fruits with BP or SS are considered to be safe for juice processing. However, in our previous research (Heinmaa et al., 2019) we found PAT in juice pressed from apples affected with BP, but not showing any visual signs of fungal infection.

The aforementioned finding led us to the hypotheses of the current study: 1) apples with physiological disorders are more susceptible to *P. expansum* infection; 2) apple juice pressed from fruits with physiological disorders has higher PAT content.

MATERIALS AND METHODS

Apple cultivars and inoculation experiments

At first, the pilot experiment was carried out in order to determine the most virulent *Penicillium* isolate out of three and to find out if there is a tendency that apples with BP symptoms are more susceptible to *Penicillium* than healthy apples. For the pilot experiment, *Penicillium spp* spores were collected from three different apple cultivars that had visible blue mould rot symptoms. Isolates were separately cultured in PDA plate for 2 weeks at 25 °C in the dark. Further on, the procedure described by Chen et al. (2017) was followed for inoculum preparation. Conidia were harvested with 0.05% Tween and counted with a hemocytometer using an optical microscope and diluted to a concentration of 1×105 conidia mL^{-1}. In the pilot experiment, 36 'Antei' apples were used: six apples with and six without BP symptoms were inoculated with three different *P. expansum* isolates. Apples were surface disinfected for 2 min with 1% sodium hypochlorite solution, rinsed three times with deionized water, and air dried on a clean bench. Each apple was inoculated in two places with 10 µL of the spore suspension (the suspension was mixed using vortexer before inoculation) using a pipette. Sterile distilled water with 0.05% Tween 20 was used as the control. Apples were stored in plastic baskets at 24 °C with RH 80%.

Lesion diameters were measured 7 and 11 days after inoculation (DAI). Two diameter values of each lesion in two mutually perpendicular directions were recorded. The average of the two values was defined as the diameter of the lesion. No apple juice was pressed in the pilot experiment. The most virulent *Penicillium* isolate out of three was used in further inoculation experiments following the same inoculation and lesion diameter measuring procedure as described above. The isolates identity as *P. expansum* was confirmed through molecular analysis as described by Adamson et al. (2015).

For further experiments, conventional 'Antei' and 'Krameri tuviõun' apples and organic 'Talvenauding' apples were harvested from South Estonian apple orchards in autumn 2017. 'Talvenauding' apples had no signs of SS and 'Antei' and 'Krameri tuviõun' had slight BP symptoms when harvested. BP symptoms develop during first two months of storage. SS symptoms usually develop during shelf life, when apples are removed from cool storage and transferred to room temperature. 100 kg of apples were harvested per cultivar and stored at 3 ± 2 °C and relative humidity 95%. The storage duration for 'Krameri tuviõun' was 12 weeks, for 'Antei' 11 weeks and for 'Talvenauding'14 weeks. Since development of BP continued during storage, 'Antei' and 'Krameri tuviõun' fruits were inspected after two months and divided into two categories: sound apples and apples with BP. Since SS develops slowly at low temperatures and rapidly at room temperature, half of the 'Talvenauding' apples were placed at 20 ± 2 °C for one week before the inoculation experiment. Flesh firmness was measured from 10 apples per category by using TMS-Pro Texture Analyser (Food Technology Corporation, USA) before inoculation. Since 'Krameri tuviõun' has conic shape and Talvenauding' is ribbed, fruit were cut in half for achieving steadiness during measurement. A small skin area was removed from two opposite sides of each fruit around the equator corresponding to the blushed and shaded sides as previously described by Saei et al. (2011). The penetration force was measured by pressing a 10 mm diameter plunger 5 mm deep into peeled fruit at a speed of 1.7 mm s^{-1}. The two readings taken on opposing sides of each fruit were averaged to obtain a mean flesh firmness value for each fruit. The readings were given in Newton (N).

In the second experiment 30 'Antei' and 30 'Krameri tuviõun' apples with and without visible BP symptoms (altogether 120 apples) were inoculated with the most virulent *P. expansum* isolate at the beginning of December 2017. Apples were stored and lesion diameters were measured as described in the pilot experiment. Half of the inoculated apples from each treatment were pressed into juice 7 DAI and the other half after 11 DAI in order to determine if and how much PAT had been produced at each time point. By 11 DAI 'Krameri tuviõun' apples were too rotten to press juice and PAT was not determined.

The third inoculation experiment was carried out at the end of January 2018 when 30 'Talvenauding' apples with and without SS symptoms were inoculated. The lesion diameters were measured 7 DAI and apples were pressed into juice on the same day. Since the lesions were already large 7 DAI, it was decided that 11 DAI the deterioration of apples was too large to press juice.

Juice pressing and patulin analyses

Non-inoculated apples with and without physiological disorders were separately pressed into juice 10 weeks after harvest. All apples with visual symptoms of fungal infection were rejected before juice pressing. 20 kg of apples in each treatment were washed and disintegrated with Voran centrifugal mill RM2.2 (Voran Maschinen GmbH, Pichl bei Wels, Austria) and pressed by water-press (WP) Lancman VSPX 120 (Gomark d.o.o., Vransko, Slovenia). Juices were pasteurized at 85 °C for 1 min by a tubular system and packed immediately into airtight 1.4-litre aluminium foil bags with Bag-in-box filler BBF6 (Gebhardt Anlagentechnik GmbH & Co. KG, Germany). Approximately 10 kg of apples for each cultivar were cut into halves in order to inspect apple cores for possible visual symptoms of fungal infection. Inoculated apples (approximately 1.5–2 kg in each category) were pressed into juice with a laboratory press Sencor Juice Extractor SJE 1005. Juices were pasteurized at 85 °C for 1 min and packed immediately into airtight 1.4-litre aluminium foil bags. PAT analyses were carried out in the Estonian Health Board laboratory, which is accredited by the Estonian Accreditation Centre. Juices were pre-treated with pectinase enzyme. The sample preparation was based on Solid Phase Extraction. PAT was eluated from the cartridge with ethyl acetate. After evaporation of the solvent, the residue was dissolved in mobile phase, and PAT was quantitatively determined by HPLC with UV detection. The level of quantification was 4 μg L^{-1}.

Statistical analysis

Data obtained in this study were subjected to statistical analysis using Dell Statistica version 13 (Dell Inc., USA) and were expressed as the means ± standard errors. The effect of *P. expansum* isolates and BP on lesion diameter was compared by analysis of variance (two-way ANOVA) (Fig. 1). Apple flesh firmness and juice PAT content were compared by one-way ANOVA (Tables 1 and 2) and significant differences among the variables for 7 and 11 DAI separately were determined according to Duncan's multiple range test ($p \leq 0.05$). Mann-Whitney U tests were performed to compare lesion diameters between categorical variables (Tables 1 and 2).

RESULTS AND DISCUSSION

In the pilot experiment, isolate 3 was the most virulent one as the average of (Fig. 1). The average effect of BP was also significant: the lesion diameters of apples with BP symptoms were significantly larger compared to apples with no disorders, which indicates that *Penicillium* develops more rapidly in apple tissues with BP symptoms.

Apple flesh firmness was not influenced by the presence of BP nor SS symptoms in any of the three cultivars studied (Tables 1 and 2). Juices pressed from non-inoculated 'Antei' apples did not contain PAT, irrespective of whether they had BP or not (Table 1). Seven DAI lesion diameters of 'Antei' apples with BP symptoms were significantly larger than the lesion diameters of sound

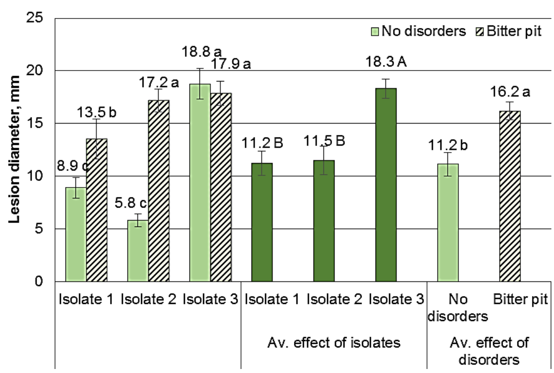


Figure 1. Mean lesion diameters of 'Antei' apples with BP symptoms and no physiological disorders inoculated with three different *Penicillium* isolates. Values with different letters for the same parameter are significantly different (two-way ANOVA).

apples. Guerrero-Prieto et al. (2017) reported that the greater the fruit Ca content, the lower the severity of *P. expansum* infection. At 11 DAI the lesion sizes were not significantly different. Among the inoculated apples, juice pressed from fruits with BP symptoms had significantly higher PAT content than juice pressed from sound apples at both 7 and 11 DAI. Also, the PAT content in the juice pressed from inoculated 'Antei' apples, which had no physiological disorders, contained PAT below the legal limit of 50 µg L^{-1}. PAT content in juice pressed from apples with BP symptoms was more than three times above the legal limit. Although no significant differences in flesh firmness were detected, the results indicated that *P. expansum* develops more rapidly and starts to produce PAT significantly sooner in 'Antei' apples with BP symptoms compared to sound apples. Since it is widely known that fungal conidia of *P. expansum* invade through wounds and bruises of the fruit (Spotts et al., 1998) it can be assumed that even though BP affected areas are below the skin, somehow the skin above disordered tissues is also weaker and makes it possible for *P. expansum* to invade the fruit.

Table 1. 'Antei' and 'Krameri tuviõun' apples' flesh firmness (N) before inoculation, mean lesion diameters of inoculated apples (mm) and juice PAT content (µg L^{-1}) ± SE; DAI = days after inoculation; BP = Bitter pit; NI = not inoculated

		'Antei'			'Krameri tuviõun'		
Dis-order	DAI	Flesh firmness, N	Lesion diameter, mm	PAT, µg L^{-1}	Flesh firmness, N	Lesion diameter, mm	PAT, µg L^{-1}
none	7	43.8 ± 1.3a	8.8 ± 0.8b	22 ± 5b	31.7 ± 0.9a	13.3 ± 1.2a	609 ± 28a
BP	7	41.3 ± 1.1a	11.1 ± 1.1a	182 ± 16a	31.2 ± 1.3a	11.0 ± 0.9a	478 ± 34b
none	11	43.8 ± 1.3A	16.1 ± 2.9A	373 ± 19B	31.7 ± 0.9A	35.0 ± 4.6A	-
BP	11	41.3 ± 1.1A	17.3 ± 2.4A	769 ± 32A	31.2 ± 1.3A	24.6 ± 3.1A	-
none	NI	43.8 ± 1.3*a*	-	< 4	31.7 ± 0.9*a*	-	< 4
BP	NI	41.3 ± 1.1*a*	-	< 4	31.2 ± 1.3*a*	-	< 4

Values with different letters for the same DAI and parameter for each cultivar are significantly different. Apple flesh firmness and juice PAT content were compared by one-way ANOVA and lesion diameters by Mann-Whitney U tests.

Table 2. 'Talvenauding' apples' fruit flesh firmness (N) before inoculation, mean lesion diameters of inoculated apples (mm) and juice PAT content (µg L^{-1}) ± SE; DAI = days after inoculation; SS = Superficial skald; NI = not inoculated

Disorder	DAI	Flesh firmness, N	Lesion diameter, mm	PAT, µg L^{-1}
none	7	39.4 ± 1.0a	23.5 ± 0.3a	4,203 ± 103b
SS	7	39.8 ± 0.6a	20.4 ± 0.6b	4,922 ± 128a
none	NI	39.4 ± 1.0A	-	< 4
SS	NI	39.8 ± 0.6A	-	162.5 ± 33A

Values with different letters for the same parameter are significantly different. Apple flesh firmness and juice PAT content were compared by one-way ANOVA and lesion diameters by Mann-Whitney U tests.

Juice pressed from non-inoculated 'Krameri tuviõun' apples with or without BP symptoms did not contain PAT. The lesion diameters of 'Krameri tuviõun' apples with and without BP symptoms were not significantly different at 7 or 11 DAI (Table 1). At 7 DAI, the content of PAT was higher in the juice pressed from initially sound inoculated apples compared to juice pressed from inoculated apples with BP symptoms. The results from ‘Krameri tuviõun’ apples were not in accordance with the findings of the other two cultivars: the lesion diameters of the apples with no physiological disorders were significantly larger and juice PAT content was higher compared to the apples with BP symptoms. The reason for that might be that ‘Krameri tuviõun’ apples were much softer than the other two cultivars studied (Tables 1 and 2) and therefore more susceptible to different competing fungi like *Botrytis* and *Gloeosporium*, which were developing alongside with *P. expansum* and suppressing its development. Earlier studies by Morales et al. (2013) have proven that interactions between *Botrytis cinerea* and *P. expansum* in grape juice medium enhanced *B. cinerea* growth and prevented PAT accumulation, which indicates that *P. expansum* is a weak competitor compared other common postharvest pathogens in apples.

‘Talvenauding’ flesh firmness was not affected by SS (Table 2). Juice pressed from apples without SS did not contain PAT over detection limit (< 4 μg L^{-1}), whereas juice pressed from non-inoculated ’Talvenauding’ apples with SS symptoms had PAT contamination of 162.5 μg L^{-1}, which is above the legislative limit of 50 μg L^{-1} (World Health Organization, 1995). At 7 DAI, apples with SS had significantly smaller lesion diameters than initially sound inoculated apples. However, PAT content was higher in the juice pressed from apples with SS symptoms than sound apples (4,922 and 4,203 μg L^{-1}, respectively). This may indicate that the lesion diameter on the fruit surface does not definitively describe the development phase of *P. expansum* and production of mycotoxin PAT. The finding is also in accordance with the results of Drusch & Ragab (2003), who reported that no correlation between the size of the lesion and the PAT concentration was found. Although ‘Talvenauding’ apples had similar flesh firmness compared to ‘Antei’ apples, the lesion diameters were approximately twice as large compared to other two cultivars and PAT content was extremely high.

Harris (2007) reported that ’Jonagold’ and ’Red Delicious’ apple juice extracted from rotten tissue of fruits inoculated with *P. expansum* in the laboratory typically contained > 1,000 μg of PAT L^{-1} by 6 days after inoculation. In our study ’Antei’ and ’Krameri tuviõun’ apple juice pressed after 7 days of inoculation contained less than 1,000 μg L^{-1} of PAT, but ’Talvenauding’ apple juice contained more than 1,000 μg L^{-1} of PAT.

Based on current study it can be stated that the lesion diameters of ‘Talvenauding’ apples affected by SS were about twice as big and the patulin content in AJ was many times higher compared to AJ pressed from ‘Krameri tuviõun’ and ‘Antei’ apples affected by BP. This indicates, that *P. expansum* develops in the fruit and starts to produce PAT faster in apples with SS compared to apples with BP. The cause for this may be because BP formation starts internally in the flesh of apples and pit appears on the fruit surface with time (Jarolmasjed et al., 2016). SS formation starts on the apple surface due to the death of cells in the epidermal or hypodermal layers of the skin (Colgan et al., 1999), which at later stages can lead to the development of internal damage and pathological disorders (Paliyath et al., 1997). Since *P. expansum* is a wound pathogen and invades the fruit through the skin, it can be assumed that SS affected fruits are more susceptible to *P. expansum* development because the surface of the fruit is already damaged.

CONCLUSIONS

It can be concluded from the current study that among the physiological disorders, SS increases the risk for PAT occurrence in juice more than BP. While the juice pressed from BP-affected apples with no visual symptoms of fungal diseases did not contain PAT, juice pressed from apples with SS had PAT content three times above the legislative limit. We also conclude that apples with physiological disorders may be more susceptible to *P. expansum* infection, but cultivar differences also play a role. In the current study, two cultivars out of three showed that if *P. expansum* infection occurred in fruit with physiological disorders, PAT production occurred significantly sooner compared to initially sound apples even though lesion diameters were not always larger. Since apples with BP and SS are often used for juice pressing all over the world, further studies with apple cultivars susceptible to these disorders should be carried out in order to increase the knowledge about the risk factors causing PAT contamination in apple juice.

ACKNOWLEDGEMENTS. This study was supported by Core Organic plus Cofund project FaVOR-DeNonDe 2015–2018 and Estonian Ministry of Rural Affairs.

REFERENCES

Adamson, K., Drenkhan, R. & Hanso, M. 2015. Invasive brown spot needle blight caused by Lecanosticta acicola in Estonia. *Scand. J. For. Res.* **30**, 587–593.

Blanco, A., Fernández, V. & Val, J. 2010. Improving the performance of calcium-containing spray formulations to limit the incidence of bitter pit in apple (Malus × domestica Borkh.) *Sci. Hortic.* **127**, 23–28.

Bryk, H. & Broniarek-Niemiec, A. 2008. Three years of experience with the apple disease control in an organic orchard. *Zemdirbyste* **95**, 395–400.

Chen, Y., Li, B., Zhang, Z. & Tian, S. 2017. Pathogenicity assay of Penicillium expansum on apple fruits. *Bio-protoc.* **7**, e2264.

Colgan, R.J., Dover, C.J., Johnson, D.S. & Pearson, K. 1999. Delayed CA and oxygen at 1 kPa or less control superficial scald without CO2 injury on Bramley's Seedling apples. *Postharvest Biol. Technol.* **16**, 223–231.

Drusch, S. & Ragab, W. 2003. Mycotoxins in fruits, fruit juices, and dried fruits. *J. Food Prot.* **66**, 1514–1527.

Fallahi, E., Conway, W.S., Hickey, K.D. & Sams, C.E. 1997. The role of calcium and nitrogen in postharvest quality and disease resistance of apples. *Hort. Science* **32**, 831–835.

Ferguson, I.B. & Watkins, C.B. 1989. Bitter pit in apple fruit. *Hortic. Rev.* **11**, 289–355.

Guerrero-Prieto, V., Berlanga-Reyes, D., Jacobo-Cuellar, J., Guigón-Lopez, C., Ojeda-Barrios, D., Ávila-Quezada, G., Núñez-Barrio, A. & Hernández-Rodríguez, O. 2017. Calcium content on apple fruit influences the severity of Penicillium expansum. *Phyton-Int. J. Exp. Bot.* **86**, 74–78.

Harris, K.L. 2007. *Patulin: surveillance in Michigan apple cider and juice, and factors influencing its production and concentration in apple products*. East Lansing, Michigan State University, PhD Thesis, 123 pp.

Heinmaa, L., Põldma, P., Mirmajlessi, S. M., Kaldmäe, H., Vangdal, E., Kidmose, U., Bertelsen, M., Lo Scalzo, R., Fibiani, M. & Moor, U. 2019. Occurrence of mycotoxin patulin and polyphenol profile of Nordic apple juices in relation to apple cultivation system and pre-processing storage temperature. *Agr. Food Sci.* **28**, 126–136.

Holb, I.J., Balla, B., Vámos, A. & Gáll, J.M. 2012. Influence of preharvest calcium applications, fruit injury, and storage atmospheres on postharvest brown rot of apple. *Postharvest Biol. Technol.* **67**, 29–36.

Jarolmasjed, S., Espinoza, C.Z., Sankaran, S. & Khot, L.R. 2016. Postharvest bitter pit detection and progression evaluation in 'Honeycrisp' apples using computed tomography images. *Postharvest Biol. Technol.* **118**, 35–42.

Lanauskas, J. & Kviklienė, N. 2006. Effect of calcium foliar application on some fruit quality characteristics of 'Sinap Orlovskij' apple. *Agron. Res.* **4**, 31–36.

Lanauskas, J., Kviklienė, N., Uselis, N., Kviklys, D., Buskienė, L., Mažeika, R. & Staugaitis, G. 2012. The effect of calcium foliar fertilizers on cv. Ligol apples. *Plant Soil. Environ.* **58**, 465–470.

McGlasson, W. 1996. Investigation into the biochemical causes of superficial scald in apples and pears – final report. Horticultural research and development corporation. The Research Arm of the Australian Horticultural Industries. Available at http://apal.org.au/wp-content/uploads/2013/11/AP111-Investigation-of-biochemical-causes-of-superficial-scald-in-apples-pears.pdf

Moor, U., Karp, K., Põldma, P., Asafova, L. & Starast, M. 2006. Post-harvest disorders and mineral composition of apple fruits as affected by pre-harvest calcium treatments. *Acta Agric. Scand. Sect. B Soil & Plant Sci.* **56**, 179–185.

Morales, H., Marín, S., Rovira, A., Ramos, A.J. & Sanchis, V. 2007. Patulin accumulation in apples by Penicillium expansum during postharvest stages. *Lett. Appl. Microbiol.* **44**, 30–35.

Morales, H., Paterson, R.R.M., Venancio, A. & Lima, N. 2013. Interaction with Penicillium expansum enhances Botrytis cinerea growth in grape juice medium and prevents patulin accumulation in vitro. *Lett. Appl. Microbiol.* **56**, 356–360.

Paliyath, G., Whiting, M.D., Stasiak, M.A., Murr, D.P. & Clegg, B.S. 1997. Volatile production and fruit quality during development of superficial scald in Red Delicious apples. *Food Res. Int.* **30**, 95–103.

Rupasinghe, H.P.V., Paliyath, G. & Murr, D.R. 2000. Sesquiterpene α-farnesene synthase: partial purification, characterization, and activity in relation to superficial scald development in apples. *J. Am. Soc. Hortic. Sci.* **125**, 111–119.

Saei, A., Tustin, D.S., Zamani, Z., Talaie, A. & Hall, A.J. 2011. Cropping effects on the loss of apple fruit firmness during storage: The relationship between texture retention and fruit dry matter concentration. *Sci. Hortic.* **130**, 256–265.

Saure, M.C. 2002. New views of the prerequisites for an occurrence of bitter pit in apple and its control by calcium sprays. *Acta Hortic.* **594**, 421–426.

Savran, H.E. & Koyuncu, M.A. 2016. The effects of superficial scald control methods having different effect mechanisms on the scald formation and α-farnesene content in apple cv. 'Granny Smith'. *Sci. Hortic.* **211**, 174–178.

Spotts, R.A., Sanderson, P.G., Lennox, C.L., Sugar, D. & Cervantes, L.A. 1998. Wounding, wound healing and staining of mature pear fruit. *Postharvest Biol. Technol.* **13**, 27–36.

Torres, E., Recasens, I., Lordan, J. & Alegre, S. 2017. Combination of strategies to supply calcium and reduce bitter pit in 'Golden Delicious' apples. *Sci. Hortic.* **217**, 179–188.

Valiuškaitė, A., Raudonis, L., Lanauskas, J., Sasnauskas, A. & Survilienė, E. 2009. Disease incidence on different cultivars of apple tree for organic growing. *Agron. Res.* **7**, 536–541.

World Health Organization. 1995. Evaluation of certain food additives and contaminants. *Techical Report Series* **859**, 36–38.

Zúñiga, C.E., Jarolmasjed, S., Sinha, R., Zhang, C., Kalcsits, L., Dhingra, A. & Sankaran, S. 2017. Spectrometric techniques for elemental profile analysis associated with bitter pit in apples. *Postharvest Biol. Technol.* **128**, 121–129.

Agronomy Research **17**(6), 2277–2285, 2019
https://doi.org/10.15159/AR.19.066

Evaluation of retention stresses of prestressing bars of a concrete ribbed panel from agricultural building after 20 years of service

M. Kiviste*, H. Lille, L. Linnus and R. Halgma

Estonian University of Life Sciences, Institute of Forestry and Rural Engineering, Chair of Rural Building and Water Management, F.R. Kreutzwaldi 5, EE51006 Tartu, Estonia
*Correspondence: mihkel.kiviste@emu.ee

Abstract. In Estonian agricultural buildings there exist a lot of precast concrete load-bearing structures, which were manufactured in the 1970s and 80s. By now, some of these are rather near for reaching their designed service life. 20 years old prestressed concrete ribbed ceiling panels (type PNS-12) with the dimensions of 6 m (length) by 1.5 m (width) from an existing agricultural building (pigsty) are the subject of current study. The objective of current study was to evaluate the retention stress of prestressing bars (PSBs) of a ribbed panel PNS-12. In other words the purpose was to find out how much of the factory-issued initial prestress was left at the PSBs after 20 years of service.

As a novel application in civil engineering strain gauges were applied in the evaluation of retention stress of PSBs in current study. The active strain gauges were glued to the opposite sides of PSBs at the middle-span of the ribbed panel, dummy gauges were glued to the unloaded steel slab. Strain gauges were connected to the half-bridge and measured with strain indicator and recorded. Retention stresses were calculated using the Hooke's law through the measured strains and elastic modulus of steel. Elastic modulus of PSBs was also measured using the universal testing machine Instron 3369 and software Bluehill 2, based on two standards.

The results demonstrated about 20.8% and 10.0% of retention stress of PSBs, respectively. The possible errors created by different aspects in experiment are also discussed.

Key words: retention stress, precast, prestressed, concrete, ribbed panel.

INTRODUCTION

Prestressed concrete is an advanced type of reinforced concrete which has been tensioned before the application of the loading. The compressive strength of concrete is nearly 10 times its tensile strength for a particular grade. Therefore, for structures which are expected to experience significant tensile loads concrete are initially prestressed during manufacture so that it is under compression. This pre-compression will ensure that the concrete section will remain under compression under the application of external tension producing forces. This is achieved by passing highly stressed (in tension) bars through the concrete section in factory. These tensile bars will take reaction from the position at which they are anchored at the ends of the concrete section thereby causing the concrete section to come under compression (Fig. 1) and, therefore, prevent tensile cracks, which characterize conventional reinforced concrete. The summarised compressive stress develop camber (the negative initial deflection) to the prestressed concrete structure.

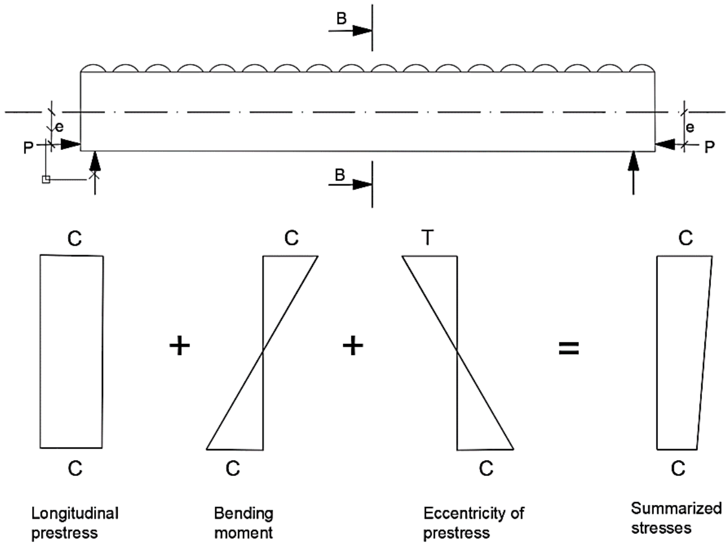


Figure 1. Principle of eccentrical prestressing. The stresses are abbreviated as follows: C – compression, T – tension.

Losses of prestress σ_p and the initial prestressing force P_0 (Fig. 2) occur in prestressing steel through several sources (Fig. 2). The losses of prestress (prestressing force) are divided as instantaneous σ_{pm0} ($P_{m,0}$; during the process of manufacture of prestressed structure) and time-dependent $\sigma_{pm\infty}$ ($P_{m,\infty}$, including long-term) (Fig. 2). The instantaneous losses of prestress σ_{pm0} ($P_{m,0}$) are divided as follows: due to a) the elastic deformation of concrete $\Delta\sigma_{p,el}$; b) fast-developing relaxation $\Delta\sigma_{p,r}$; c) loss of anchorage $\Delta\sigma_{p,r}$; d) temperature change in prestressing steel $\Delta\sigma_{p,temp}$ and e) friction $\Delta\sigma_{p,\mu}$. The time-dependent losses of prestress $\sigma_{pm\infty}$ ($P_{m,\infty}$) are divided as follows: due to a) elastic deformation of concrete $\Delta\sigma_{p,el}$; b) loss of anchorage $\Delta\sigma_{p,r}$; c) temperature change in prestressing steel $\Delta\sigma_{p,temp}$; d) friction $\Delta\sigma_{p,\mu}$ and e) creep and shrinkage of concrete and relaxation of steel $\Delta\sigma_{p,c+s+r}$.

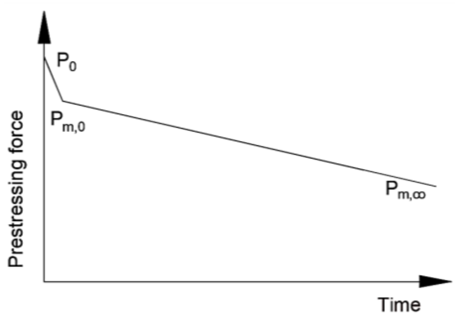


Figure 2. Principle of losses in prestressing force (P_0) in time.

Losses of prestress can be evaluated analytically and experimentally. The experimental techniques used to evaluate the losses of prestress include several typologies (Azizinamini et al., 1996; Labia et al., 1997; Baran et al., 2005; Wu et al., 2011; Caro et al., 2013).

In Estonian industrial and agricultural buildings there exist a lot of precast concrete load-bearing structures, which were mass-produced in concrete factories of the former Soviet Union from the 1960ies until at least 1990.

In this research precast concrete ribbed ceiling panels from an existing agricultural building (pigsty) are studied. These kind of precast concrete ceiling panels were very commonly used in the industrial, agricultural and military buildings across the former Soviet Union and are partially still in service in Eastern European and Baltic countries. By now, due to corrosion deteriorations, many of these ribbed panels are rather near for reaching their designed service life. The owners of the building need to make informed decision whether to further use, refurbish or demolish those ribbed panels.

There is no evidence-based information how much retention stress is left in prestressing bars (PSBs) of ribbed panels after long period (many decades) of service. The objective of this study is to evaluate experimentally the retention stress of PSBs of a prestressed ribbed panel PNS-12. In other words the purpose was to find out how much of the factory-issued initial prestress was left at the PSBs of ribbed panels after 20 years of service.

MATERIALS AND METHODS

The studied ribbed panels were from Vara pigsty (Tartu county, Estonia), which was constructed in 1986, and the ribbed panels were tested in 2006. The top view, longitudinal and transverse section of a ribbed panel PNS-12 is demonstrated in Fig. 3. The abbreviation PNS denote that the ribbed panels are prestressed and the number refer to different load-bearing capacities (PK-01-111, 1961).

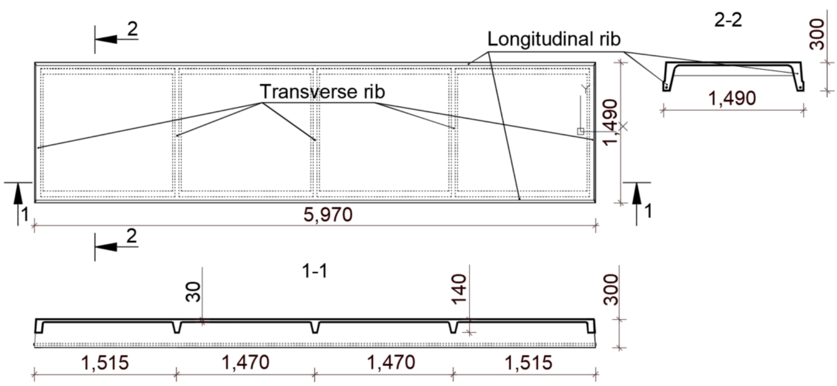


Figure 3. Top view, longitudinal and transverse section of a ribbed panel PNS-12 (PK-01-111, 1961). *Dimensions are in mm.*

Six ribbed panels were assessed on a 6-point rating scale (5, 4, 3, 2, 1 and 0) according to visually distinguishable corrosion deterioration. Grade 6 indicates no corrosion deteriorations, grade 2 indicates cracks in transverse ribs, grade 1 cracks in longitudinal ribs and grade 0 spalled cover of the longitudinal rib of a ribbed panel (Miljan & Kiviste, 2010). Fig. 4 shows the corrosion deteriorations of ribbed panel P8. Due to corrosion-affected spalled cover at the longitudinal ribs, ribbed panel P8 received grade 0 in the visual rating scale.

Five 20 years old prestressed concrete ribbed ceiling panels of type PNS-12 from an existing agricultural building (pigsty) are studied. The prestressing and reinforcing steel details of a ribbed panel PNS-12 are shown in Fig. 5.

According to the design drawings of ribbed panels PNS-12 employed two hot-rolled low-alloyed PSBs of mark of mark 35 GS (C from 0.3 to 0.37%, Mn from 0.8 to 1.2%, Si from 0.6 to 0.9%, Cr = 0.3%, Ni = 0.3%, Cu = 0.3% (PK-01-111, 1961; GOST 5058-65). The diameter and prestress of PSBs of ribbed panels PNS-12 are 16 mm and 343 MPa (N mm^{-2}), respectively. The ultimate strength of pre-stressing steel of PNS-12 should be at least 5,500 kgf cm^{-2} (539 MPa) to correspond to its mark. The prestressing and reinforcing steel details of ribbed panels PNS-12 are shown in Fig. 5.

Figure 4. The condition of ribbed panel P8 before testing. Due to corrosion-affected spalled cover at the longitudinal ribs, ribbed panel P8 received grade 0 in the visual rating scale.

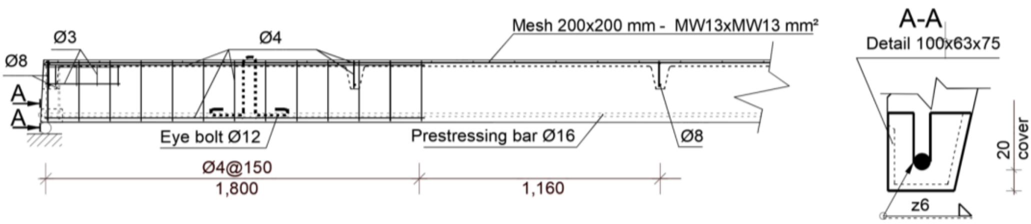


Figure 5. Prestressing and reinforcing steel details of the ribbed panel PNS-12 (PK-01-111, 1961). *Dimensions are in mm.*

A total of twelve PSB specimens with a length 300mm were cut from longitudinal ribs of each studied ribbed panel (P7-P11). The yield strength, ultimate strength and modulus of elasticity of each PSB specimen was determined in tensile test using testing machines P-20 and Instron 3369. The length change for calculating the modulus of elasticity was measured by an optical gauge (Advanced Video Extensometer 2663-821) and software Bluehill 2, which is based on the American (ASTM E8-16a) and European (EVS-EN ISO 6892-1:2016) standards. For modulus of elasticity the approximation line was obtained between the loads (initial F_1 and final F_2) as 10% and 40% of yield strength. The diameter of PSB was calculated according to standard EVS-EN ISO 6892-1:2016.

For the evaluation of compressive strength, ten concrete cores with a diameter of 50 mm were drilled (steel drill with diamond bits Rems Picus S3) from the longitudinal rib of ribbed panels. Later on, the cores were cut to the height of also 50 mm, in order to reach the height and diameter ratio of 1, which is equivalent to the standard cube strength (EVS-EN 13791:2007). The compressive strength of concrete of ribbed panels PNS-12 should correspond to concrete strength mark M200 = 200 kgf cm^{-2}, which corresponds to 19.6 MPa (PK-01-111).

One ribbed panel (P7) with a largest camber (negative initial deflection, -20 mm) and least corrosion deteriorations (grade 3) was chosen to the retention stress evaluation procedure. Ribbed panel was placed upside down in order to prevent the additional stresses developed by the dead-weight of a ribbed panel.

A first attempt was made to evaluate the longitudinal change of length of prestressed bars with Helios Preisser Digi-Met 0 273 7 Digital Caliber 450 mm with an accuracy of 0.01 mm. However, the accuracy of the caliber applied in measurements was not sufficient, and was used only for approximate evaluation later on.

The retention stress of prestressed bars was evaluated at the middle-span of the two longitudinal ribs (A-B and C-D) of a ribbed panel (P7). In those places the concrete was locally removed. As a novel application in civil engineering, strain gauges were applied in the evaluation of retention stress of PSBs. Strain gauge is based on the physical property of electrical conductance and its dependence on the conductor's geometry. The measured electrical resistance of the strain gauge is proportional to the amount of induced strains.

Strain gauge with a basic length 20 mm, resistance 200 W and a gauge factor 2.0 at 20 ± 1 °C was applied for measuring strains. Strain gauges were glued (БФ-2 glue) in the longitudinal direction onto pre-cleaned and flat-sanded surface (the finish roughness of the surface (about 3.2 μm) was obtained with sandpaper) of PSB and joined to form of half-bridge with dummy gauge glued to the unloaded steel plate, which was needed for thermal compensation (Fig. 6).

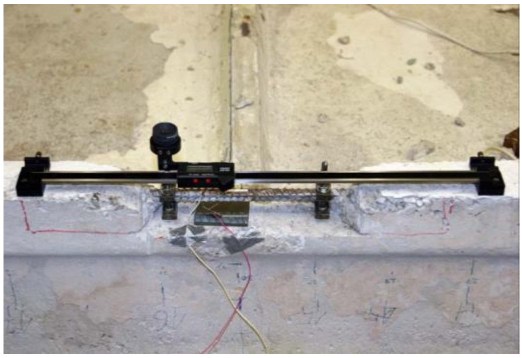


Figure 6. Pre-cleaned and pre-prepared PSB, with strain gauges and dummy gauge on the steel plate. Attached Helios Preisser Digi-Met 0 273 7 Digital Caliber 450 mm had insufficient accuracy (0.01 mm) and was applied only for approximate evaluation of longitudinal change of length of PSB.

After cutting the PSB the signals of strain were recorded by strain indicator (Wemmo-Anti), consisting of a half bridge and a processor which is capable of saving signals and saved data can be sent directly to computer for further processing. Calibration of strain gauges represent separate experiments. The strain gauges were glued onto a cantilever beam of uniform strength, which was loaded step by step mechanically by a screw. The readings of the strain and deflection indicators (dial gauge with accuracy of 0.002 mm) were registered at every step. The strain indicator constant was found by using the program *MS Excel 2016* with the regression analysis function. As a result, the strain indicator constant $C = 5.01\times10^{-7}$ (per unit of the strain indicator) was obtained.

RESULTS AND DISCUSSION

The material properties of the studied ribbed panels were found. The results of tensile test of PSB specimens averaged on studied ribbed panels (P7-P11) are presented in Table 1. The tensile test graph of two randomly chosen PSB specimens from each of the studied ribbed panel, thus, a total of ten specimens are presented in Fig. 7. All the studied prestressed bars exhibited yielding as show in Fig. 7.

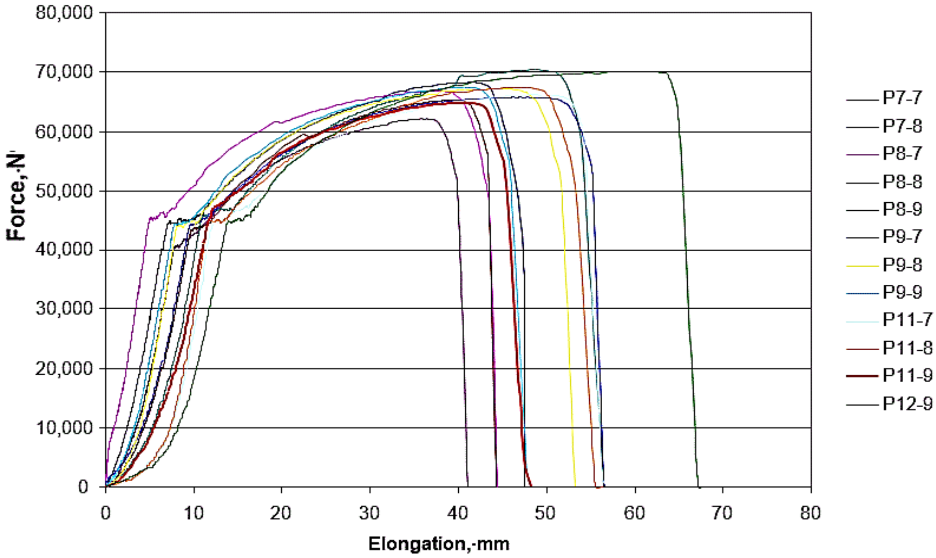


Figure 7. A graph representing the tensile test of two randomly chosen PSB specimens from each studied ribbed panel (P7-P11).

According to construction drawings, ribbed panels PNS-12 employed two hot-rolled low-alloyed PSBs of mark 35 GS. The ultimate strength of pre-stressing steel of ribbed panels PNS-12 should be at least 539 N mm^{-2} to correspond to its mark (PK-01-111, 1961; GOST 5058-65).

Table 1. Tensile test results of the studied PSB specimens (ø16 mm) from ribbed panels PNS-12 (P7-P11). The ribbed panel for the evaluation of retention stresses (P7) is marked as bold

Ribbed panel	Average ultimate strength of PSB specimens, MPa	Average yield strength of PSB specimens, MPa	Average modulus of elasticity of PSB specimens, GPa
P7	**657.1**	**544.3**	**197.0**
P8	*698.9*	620.5	*192.0*
P9	649.0	576.2	221.5
P10	635.8	576.1	175.7
P11	660.6	566.0	174.3

The average ultimate strength of PSB specimens of the studied ribbed panels (in Table 1) was found higher than strength mark. Thus, the ultimate strength of prestressing steel was found to conform the requirements of prestressed ribbed panels PNS-12. However, the characteristic ultimate strength of high-strength wire strands applied in modern prestressed concrete is f_{pk} = 1,500…2,000 MPa (N mm^{-2}, EVS 833-1:2002, EN 10138-1: 2000). Therefore, the strength properties of the studied prestressing steel bars of ribbed panel PNS-12 are about three times lower and thus directly incomparable to high-strength wire strands. Therefore, also much greater losses of prestress due to both fast-developing and long-term relaxation are expected in the studied PSBs.

The results of compressive test of cores from the studied ribbed panels is presented in Table 2. According to construction drawings of ribbed panels PNS-12 the average compressive strength of concrete should correspond to concrete strength mark M200, which corresponds to 19.6 MPa (PK-01-111). Table 2 shows that the average compressive strength of cores from the studied ribbed panel (P7, which was used for evaluation of retention stresses) was 44.3 MPa. Thus, the concrete compressive strength of ribbed panel P7 was more than twice greater than required (strength mark) and has not decreased during 20-years time. For the study of losses of prestresses also concrete strains have to be studied in order to evaluate the instantaneous and time-dependent (shrinkage and creep) strains of concrete. However, that was impossible to perform in current study as comparative (laboratory) study of concrete specimens at the different age would be necessary for that. The instantaneous and time-dependent strains (including shrinkage and creep strains) of concrete prismatic specimens over 1 year were studied in different research (Caro et al. (2013)).

Table 2. Core strength, carbonation and cover depth of concrete of the studied ribbed panels PNS-12 (P7-P11). The ribbed panel for the evaluation of retention stresses (P7) is marked as bold

Ribbed panel /Longitudinal rib	Average of core compressive strength, MPa	Standard deviation of core compressive strength, MPa	Carbonation depth / cover depth of concrete, mm
P7/A-B	**44.3**	**± 4.8**	**7.0 / 23.6**
P7/ D-C			**7.4 / 24.2**
P8/A-B	*18.4*	± 4.9	*27.0 / 24.4*
P8/ D-C			*22.3 / 24.3*
P9/A-B	56.4	± 3.8	8.4 / 25.0
P9/ D-C			8.4 / 24.2
P10/A-B	43.7	± 6.8	9.9 / 25.6
P10/D-C			9.4 / 27.7
P11/A-B	45.8	± 8.7	8.1 / 24.3
P11/D-C			9.1 / 24.6

The ribbed panel (P7) with the least corrosion deteriorations was deliberately chosen for the study as the corrosion deteriorations increase the losses of prestress. The studied ribbed panel (P7) received grade 3 at visual scale, which means no visual corrosion deteriorations were found at the longitudinal ribs. The full cover has to be carbonated (Table 2) for the propagation of carbonation-induced corrosion. However, only about one third of a cover was carbonated at the studied ribbed panel (P7). In comparison, the ribbed panel with the lowest compressive strength of concrete (P8 in Table 2) had also the full cover carbonated in average. Also almost full cover carbonation could lead to corrosion. Also, the cleaned out PSB (in Fig. 6) had also no signs of corrosion.

The ultimate strength, yield strength and modulus of elasticity (E-modulus in Table 1) of PSB specimens of studied ribbed panel (P7) for calculations was found as 657.1, 544.3 and 197×10^3 MPa (N mm^{-2}), respectively. The retention stresses of PSBs were calculated using the Hooke's law: $\sigma = E \cdot \varepsilon$, where $\varepsilon = C(A'' - A')$, A" is the final reading of strain indicator, after the cutting of PSBs and A' is initial reading of strain indicator before cutting.

The retention stress at the middle-span of longitudinal rib A-B of ribbed panel (P7) are presented as follows: A'' = 945 unit, A' = 224 unit and σ = 71.2 MPa (N mm^{-2}) The retention stress of the longitudinal rib C-D of a ribbed panel (P7) is presented as follows: A'' = 756 unit, A' = 412 unit and σ = 34.2 MPa (N mm^{-2}). The retention stresses of PSBs at two longitudinal ribs of a same ribbed panel differ, but the difference is less than a magnitude.

The initial prestressing force P_0 and the initial prestress σ_p of the studied ribbed panel (P7) is not known. Unfortunately, P_0 and σ_p are also untraceable as a part of a batch of precast prestressed ribbed panels in mass-production in concrete factory. Therefore, the actual loss of prestress of the individual ribbed panel could not be found. According to construction drawings the prestress of PSBs of ribbed panels PNS-12 is 343 N mm^{-2} (3,500 kgf cm^{-2}, PK-01-111, 1961). Therefore, the retention stresses are 20.8% (longitudinal rib A-B) and 10.0% (C-D) of the assumed initial prestress. This means about 79–90% loss of prestress of a ribbed panel at the age of 20 years.

In comparison with other experimental research, 25–60% measured losses of prestress were noticed already over 1 year (Caro et al., 2013). Caro et al. (2013) evaluated the losses of prestress in prestressed concrete prismatic specimens of three different cross sections The total measured losses of prestress values ranged from 25% to 60%: 25–40% for specimens with greater cross-sections (100×100 mm^2), 40–50% for specimens with intermediate cross-sections (80×80 mm^2), and 50–60% for specimens with smaller cross-sections (60×60 mm^2).

The determination of losses of prestress usually involves complicated, laborious procedures because time-dependent prestress losses are inter-dependent (Francis & Au, 2011). Prestressing reinforcement relaxation is continuously altered by changes in stress due to concrete shrinkage and creep. Concrete creep, in turn, constantly alters by changes in prestressing steel stress. Moreover, concrete shrinkage and creep movements are partially restrained by the prestressing steel.

It is generally accepted that losses of prestress have little effect on ultimate design strength and on the capacity of pretensioned concrete structures, but can affect service conditions (ACI 318-11). It has been found out in earlier studies that the residual load-bearing flexural capacity of the studied six ribbed-panels was sufficient. All the studied panels, (with ultimate load q_u), irrespective of their grade were able to carry the design load q_d (q_u/q_d. > 1.95) (Kiviste & Miljan, 2010).

CONCLUSIONS

Retention stresses of the 20-year-old prestressed ribbed ceiling panel were experimentally investigated with the strain gauges. For the background information of losses of prestress the strength properties of concrete and prestressing steel bars of five ribbed panels were also evaluated.

The studied prestressing bars have lost most of its initial prestresses and the retention stresses are relatively low (10% and 21%). Although conforming to the requirements, the strength properties of the studied PSBs was greatly lower than that of prestressed wire stands applied in modern prestressed concrete. Therefore, also much greater losses of prestress were found in the studied PSBs.

The residual load-bearing capacity of the ribbed panel evaluated in earlier study was found to be sufficient. Thus, the studied structure was still in accordance with the ultimate limit state. However, the losses of initial prestress (90% and 79%) are indicating that the studied structure (ribbed ceiling panel) at the age of 20 years is inefficient according to service conditions (serviceability limit state). Therefore, careful monitoring of the studied structure is required in the further operation. Undesired cracking or excessive deflections may occur and could impair the serviceablity of a structure in the future.

REFERENCES

ACI E8/ E8M - 16a. 2016. *Standard Test Methods for Tension Testing of Metallic Materials*. ASTM International, West Conshohocken, PA, 30 pp.

ACI 318–11. 2011. ACI Committee 318. *Building code requirements for reinforced concrete*. Farmington Hills, MI: American Concrete Institute, 509 pp.

Azizinamini, A., Keeler, B.J., Rohde, J. & Mehrabi, A.B. 1996. Application of a new non-destructive evaluation technique to a 25-year-old prestressed concrete girder. *PCI Journal* **41**(3), 82–95.

Baran, E., Shield, C.K. & French, C.E. 2005. A comparison of methods for experimentally determining prestress losses in pretensioned prestressed concrete girders. In: *Historic innovations in prestressed concrete.* Russell, B.W., Gross, S.P. (Eds.) American Concrete Institute, Farmington Hills, MI, pp. 161–179.

Caro, L.A., Marti-Vargas, J.R. & Serna, P. 2013. Prestress losses evaluation in prestressed concrete prismatic specimens. *Engineering Structures* **48**, 704–715.

EVS 833-1:2002. Prestressing steels. Part 1: General requirements. European Centre for standardization, 24 pp.

EN 10138-1: 2000 Prestressing steels. Part 1: General requirements. European committee for standardization (CEN), 21 pp.

EVS-EN ISO 15630-3:2010. Steel for the reinforcement and prestressing of concrete – Test methods – Part 3: Prestressing steel. Estonian Centre for Standardisation, 34 pp. [in Estonian].

EVS-EN ISO 6892-1:2016. Metallic materials - Tensile testing - Part 1: Method of test at room temperature (ISO 6892-1:2016). Estonian Centre for Standardisation, 77 pp.

EVS-EN 13791:2007. Assessment of in-situ compressive strength in structures and precast concrete components. Estonian Centre for standardisation, 30 pp.

Francis, T.K. & Au, X.T. 2011. Accurate time-dependent analysis of concrete bridges considering concrete creep, concrete shrinkage and cable relaxation. *Engineering Structures* **33**, 118–126.

GOST 5058-65 Low-alloyed structural steel. Marks and general technical requirements. Moscow. 1965. 10 pp. (in Russian).

Kiviste, M. & Miljan, J. 2010. Evaluation of residual flexural capacity of existing pre-cast pre-stressed concrete panels—A case study. *Engineering Structures* **32**(10), 3377–3383.

Labia, Y., Saiidi, M.S. & Douglas, B. 1997. Full-scale testing and analysis of 20-year-old pretensioned concrete box girders. *ACI Structural Journal* **94**(5), 471–482.

Miljan, J. & Kiviste, M. 2010. Estimation of residual flexural capacity of existing precast concrete panels by visual inspection. *Agronomy Research* **8**, 177–191.

Series PK-01-111. 1961. P*re-stressed concrete ceiling panels of dimensions 1.5 by 6m. Construction drawing*s. Moscow: Central institute of typical design drawings, 20 pp. (in Russian).

Wu, C. H., Zhao, W., Beck, T. & Peterman, R. 2011. Optical sensor developments for measuring the surface strains in prestressed concrete members. *Strain* **47**, 376–386.

Agronomy Research **17**(6), 2286–2294, 2019
https://doi.org/10.15159/AR.19.203

Objective organoleptic, structural-and-mechanical parameters of vegetables depending on their degree of ripeness

K. Kostetska*, N. Osokina, H. Gerasymchuk and O. Nakloka

Uman National University of Horticulture, Department of Technology of Storage and Processing of Grain, 1 Instytutska street, UA20305 Uman, Ukraine
*Correspondence: kostetskakateryna@gmail.com

Abstract. Organoleptic, structural-and-mechanical indicators determine the suitability of fruiting vegetables for harvesting and preservation, as well as the ratio of separate parts of the fruit. All these indicators affect the quality of products.
Experimental work was carried out in the conditions of the laboratory of the Department of Technology of Storing and Processing of Grain. A complex of organoleptic, commercial, physical and thermophysical indicators of eggplant, sweet pepper and tomato fruits were developed in the work to determine the time of their harvesting.
It was determined that physical density and mechanical strength in the fruits of sweet pepper of technical degree of ripeness were 6% higher than in the fruits of biological degree of ripeness.
Peculiarities of the ripeness degree significantly affected the amount of inedible part of the fruit (seeds, seed cavity and peduncle), which was 1.2 times less in sweet pepper fruits of technical degree of ripeness than biological degree of ripeness.
Considerable varietal difference of eggplant fruits by the amount of edible and inedible parts of the fruit was determined. Peculiarities of the variety also significantly affected the density and hardness of the fruit.
Red tomatoes fruits of Iskorka variety had tender pulp consistency and relatively low fruit density (0.88 g cm^{-3}) and mechanical strength (3.00 kg cm^{-2}).
The objective organoleptic, structural-and-mechanical indicators of fruiting vegetables were determined depending on their degree of ripeness; to determine the optimal time of harvesting the fruits of eggplant, sweet pepper, tomato.

Key words: organoleptic indicators, structural-and-mechanical indicators, variety, degree of ripeness, vegetables.

INTRODUCTION

The main conditions for reducing the loss of fruit and vegetable products are timely harvest, as well as developing and improving the methods of storage. Every year, many products lose their food value because of improper storage, and sometimes it is completely spoiled. One should store food in accordance with scientifically established parameters to ensure that vegetables meet the best biochemical and organoleptic characteristics for a long time.

In a context characterized by a growing consumer's interest in locally produced foods, the safeguard of widely known fruit varieties appears relevant (Darby et al., 2008; Denver & Jensen, 2014; Bartolini & Ducci, 2017).

In all countries of the world, about 247 kinds of vegetables are consumed, 40 of which are consumed Ukraine while 70 are produced in the neighboring countries. Ukraine occupies a leading place in the production of vegetables and fruits among the neighboring countries (Overchenko, 2005). The population has increased interest in consuming exclusively natural food as a way of improving the quality of life (Corbo et al., 2006; Osadcuks & Pecka, 2016). Now both professionals and consumers talk about natural products with special properties (Philipchuk, 2005).

Vegetable ripeness is characterized by such organoleptic characteristics as condition of skin, pulp, and their color according to current standards. Thus, the internal anatomical structure characterizes fruits ripeness and is indicated in the standards as a seed cavity with ripe seeds (sweet pepper of biological degree of ripeness, red tomatoes) or with non-ripe white seeds (sweet pepper and eggplants of technical degree of ripeness), pulp of various density, but not over-ripe. However, these indicators are subjective because the fruits of different varieties have their own peculiarities (Lima et al., 2005; Freitas & Costa, 2006; Leccese et al., 2012).

MATERIALS AND METHODS

The aim of the present work was to determine the objective organoleptic, structural-and-mechanical indicators of fruiting vegetables depending on their degree of ripeness and to determine the optimal time of harvesting the fruits of eggplant, sweet pepper, tomato.

Research Methodology (STN 2659–94, 1995; STN 2660–94, 1995; STN 3246–95, 1997; Rubatzky, et al., 1997; Yeshchenko, et al., 2005).

The area of experimental plots under tomatoes, sweet pepper and eggplants was 20 m^2 each. Repeatability of the experiment was three-times. Planting of seedlings in the age of 60 days was carried out in mid May, when the threat of freezing completely ceased, in the open ground according to the scheme of 70×20 cm. Technological measures were carried out in accordance with the requirements of the crop. Phenological observations – by beginning and passing of the phenological phases of plants development.

The duration of phases of plants vegetation was determined under different weather conditions. The following indicators of fruiting vegetables were defined: objective organoleptic – by form, colour, taste, smell; – internal anatomical structure and biometric – by weight, length, diameter, width, volume of fruit, number of fruits per plant, number of commodity fruits per plant, inedible part of the commodity weight of the fruit. Sample weight for the experiment was 5,000 g.

Hardness of the fruit covering tissues was determined by a penetrometer FT 327 with a plunger of 11 mm in diameter (pre-cutting the skin).

Volume of fruit was assessed by submerging them into water in a measuring cylinder. Volumetric (bulk) weight of vegetables in the volume of 1 m^3 provided free laying, taking into account free space between individual specimens of eggplant fruits, sweet pepper and tomatoes, was determined using a box with dimension of 1 m each side making the volume 1 m^3. The box was filled to the edges; the mass of fruits was estimated by the difference between the weight of the box and products.

Physical density (specific weight) was calculated by the formula:

$$P_{\phi} = \frac{\text{м}}{V}, \qquad (1)$$

where P_{ϕ} – physical density, kg m^{-3}; м – product weight, kg; V – volume of product, m^3.

Organoleptic evaluation of the quality of fresh fruit was carried out using a five-point system (STN 87561–79, 1979).

Statistical processing of research results was carried out using special program packages (Excel, Statistics). Differences were considered to be significant at validity of $\alpha = 0.95$.

RESULTS AND DISCUSSION

Fruits of sweet pepper of technical and biological degree of ripeness, tomato of green, flesh-colored, brown, pink, red (yellow) degree of ripeness, while eggplant only of technical degree are being used for harvesting, sale, processing and storage. Properties of vegetables of different degrees of ripeness affect their nutritional value, storage capacity and marketable quality.

Objective indicators of optimum degree of fruits ripeness of eggplant, sweet pepper and tomato include colour, appearance, anatomical structure of fruits, amount of seeds, share of edible and inedible part of the fruit, their weight and volume, hardness and density.

Table 1 shows organoleptic characteristics by shape, colour, taste and smell of eggplant fruits of Helios and Almaz variety, tomatoes of Iskorka variety, sweet pepper of Novohohoshary variety of different degrees of ripeness, as well as characteristics of their inner anatomical structure.

Table 1. Objective organoleptic indicators of fruiting vegetables depending on the variety and degree of ripeness

<table>
<tr><td rowspan="5">Indicator</td><td colspan="2">Eggplant</td><td colspan="2">Sweet pepper</td><td>Tomatoes</td></tr>
<tr><td colspan="5">variety</td></tr>
<tr><td>Almaz</td><td>Helios</td><td colspan="2">Novohohoshary</td><td>Iskorka</td></tr>
<tr><td colspan="5">degree of ripeness</td></tr>
<tr><td colspan="2">technical</td><td>technical</td><td>biological</td><td>red</td></tr>
<tr><td>Shape</td><td>cylindrical</td><td>ball-like</td><td colspan="2">ribbed-and-round</td><td>oval</td></tr>
<tr><td>Colour</td><td>dark-violet</td><td>light-violet</td><td>green</td><td>red</td><td>red</td></tr>
<tr><td>Taste and (or) smell</td><td>peculiar without a foreign smell</td><td>peculiar without any foreign smell, with no strong solanaceous smell</td><td>sweet taste with slight flavour strength; with peculiar flavour</td><td>sweet taste with tender flavour strength; peculiar, strongly pronounced aroma</td><td>taste and (or) smell</td></tr>
<tr><td>Inner-anatomical structure</td><td colspan="2">dense pulp without free space, greenish or white, seed cavity with unripe white seeds</td><td>seeds of wax or milk ripeness</td><td>ripe seeds</td><td>tender consistency of pulp, ripe seeds</td></tr>
</table>

Vegetative phases duration of plants under certain agro climatic conditions was established to supplement objective indicators of the harvesting period of tomatoes, eggplants, sweet peppers for further processing. The beginning and duration of the vegetation phases of eggplant, sweet pepper and tomato plants were established according to the indicators given in Tables 2–4.

Table 2. Vegetation period of eggplant plants of Helios and Almaz varieties

No.	Phase	Duration
1.	Term of planting	From the 21st to 31st days of May
2.	Formation of flower buds	From the 1st to 10th days of June
3.	Beginning of blossom	From the 21st to 30th days of June
4.	Mass blossom	From the 11st to 31st days of July (up to 16 flowers per plant)
5.	Beginning of fruit-set	From the 1st to 10th days of July
6.	Ripe	15–20 days after blossom
7.	First formed fruits	From the 11st to 20th days of July
8.	Mass fruit bearing	From the 21st of July to the 10th day of August
9.	Period of vegetation[1]	131 ± 10 days
10.	Duration of fruits being under technical degree of ripeness	to 34 days

Note. [1] – harvest time is up to 63 days.

Table 3. Vegetation period of sweet pepper plants of Novohohoshary variety

No.	Phase	Duration
1.	Term of planting	From the 21st to 31st days of May
2.	Formation of flower buds	From the 1st to 10th days of June
3.	Beginning of blossom	From the 11st to 30th days of June
4.	Mass blossom	From the 1st to 31st days of July
5.	Beginning of fruit-set	From the 1st to 10th days of July
6.	Ripe	10 ± 2 days after blossom
7.	First formed fruits	From the 11st to 20th days of July
8.	Mass fruit bearing	From the 21st of July to the 10th day of August (35–40 days since ovary appearing)
9.	Period of vegetation[1]	125 ± 10 days (144 ± 10 days)
10.	Duration of fruits being under technical degree of ripeness	to 37 days

Note. [1] – fruits of technical (biological) degree of ripeness with the duration of harvest of 72 days.

Table 4. Vegetation period of tomatoes plants of Iskorka variety

No.	Phase	Duration
1.	Term of planting	From the 21st to 31st days of May
2.	Formation of flower buds	From the 1st to 10th days of June
3.	Beginning of blossom	From the 11st to 30th days of June
4.	Mass blossom	From the 1st to 31st days of July
5.	Beginning of fruit-set	From the 21st to 30th days of June
6.	Ripe	10 ± 2 days after blossom
7.	First formed fruits	From the 21st of June to the 10th day of July
8.	Mass fruit bearing	From the 21st of July to the 10th day of August
9.	Period of vegetation	70 ± 6 days

According to the observation, it was found that individual phases: term of planting (last 10 days of May), formation of flower buds (first 10 days of June), mass fruit bearing (last 10 days of July to first 10 days of August) occurred in the same terms regardless of the type of vegetables.

In particular, according to the phenological observations, the phase of plants floral bud formation of eggplant of Almaz variety came 12 days after planting the seedlings, and after 15 days in Helios variety. Thus, the difference between the dates of beginning of the phase of flower bud formation of the experimental varieties was three days.

Eggplant differed in slightly later period of flowering. Instead, the phase of beginning of fruit-set in tomato was at the end of June, while eggplant and sweet pepper began setting fruit in July.

Ripening of eggplant fruits occurred within 15–20 days after flowering, while pepper and tomato – 10 ± 2 days after flowering. The first formed tomatoes were observed in last 10 days of June to first 10 days of July, sweet pepper and eggplant – in mid-10 days of July. The duration of fruits being in the degree of ripeness of eggplant varieties of Helios and Almaz was up to 34 days whereas this duration was 37 days for sweet pepper of Novohohoshary variety.

The harvest frequency and duration of harvest of eggplant and sweet pepper depend on the terms of planting, growing conditions and biological characteristics of the variety. Duration of the harvest period in the studied varieties of eggplant and sweet pepper was up to 63 days and 72 days, respectively.

It was found that the period of plants vegetation was 131 ± 10 days for eggplant, 125 ± 10 days for sweet pepper and 70 ± 6 days tomato. We defined commodity state, structural and qualitative indicators of fruiting vegetables. The shape of vegetables is characterized by length, width and diameter. The research showed that these indicators had varietal characteristics (Table 5).

Table 5. Structural-and-mechanical properties of fruits of eggplant plants

Indicator	Variety		LSD_{05}
	Almaz	Helios	
Length without peduncle, cm	11.30	8.20[1]	*0.49*
Weight of one commodity fruit (g)	144.50	210.80	*8.90*
Volume of fruit (cm^3)	110.50	186.60	*7.44*
Physical solidity (g cm^{-3})	1.31	1.13	*0.06*
Density (kg cm^{-2})	7.90	7.20	*0.38*
Number of fruits on one plant (pcs)	6–9	4–8	*0.3*
Number of commodity fruits on one plant (pcs)	4–8	2–5	*0.2*

Note. [1] – diameter, cm.

Eggplants of Helios variety had a ball-like shape of fruits with an average length of 8.2 cm, and Almaz variety had cylindrical fruit shape with an average length of 11.3 cm.

Average weight of eggplants of the technical degree of ripeness of Almaz variety was 144.5 g, which was 66.3 g or 32% less than Helios variety. Volume of eggplants of the technical degree of ripeness of Helios variety was 186.6 cm^3, which was 76.1 cm^3 or 41% greater than Almaz variety.

Eggplants of Almaz variety had 6–9 fruits on one plant, of which 4–8 pcs correspond to standard (sample) (STN 2660–94, 1995). Eggplant of Helios variety had 1–3 fruits more than Almaz variety.

Structural-and-mechanical and physical properties of sweet pepper fruits are given in Table 6. Six to 12 fruits were harvested from one sweet pepper plant, and 5–8 pcs correspond to standard (STN 2659–94, 1995).

Table 6. Structural-and-mechanical properties of sweet pepper fruits of Novohohoshary variety

Indicator	Degree of ripeness		LSD_{05}
	technical	biological	
Length without peduncle (cm)	7.30	7.45	*0.37*
Width (cm)	5.41	5.70	*0.28*
Thickness of walls (cm)	0.60	0.65	*0.03*
Weight of one commodity fruit (g)	100.50	103.00	*5.09*
Volume of fruit (cm^3)	98.04	105.80	*5.10*
Physical solidity (g cm^{-3})	1.03	0.97	*0.05*
Density (kg cm^{-2})	8.60	8.10	*0.42*
Number of fruits on one plant (pcs)	6–12		
Number of commodity fruits on one plant (pcs)	5–8		

Fruits of sweet pepper of Novohohoshary variety of technical degree of ripeness differed in structural indicators compared with the fruits of biological degree of ripeness. Thus, average length of peppers of technical degree of ripeness was less by 2%, width by 5%, weight by 2.4%, volume by 7.3% than the fruits of biological degree of ripeness and were 7.3 and 5.4 cm, 100.5 g, 98.0 cm^3, respectively.

Variation-and-statistical data processing in Table 5 indicated that the peculiarities of eggplant fruits did not affect their biometric and anatomical characteristics but had a significant effect on density and physical solidity. The reliable difference by the degree of ripeness was observed only for the indicators of length and weight of pepper fruits of Novohohoshary variety (Table 6).

Structural-and-mechanical and physical properties of tomato fruits are presented in Table 7.

Table 7. Structural-and-mechanical properties of tomatoes fruits of Islorka variety

Indicator	Year				LSD_{05}
	2007	2008	2009	average	
Diameter (cm)	5.22	5.48	5.30	5.30	*0.27*
Weight of one commodity fruit (g)	92.65	93.25	93.10	93.00	*4.66*
Volume of fruit (cm^3)	104.10	106.00	105.60	105.50	*5.28*
Physical solidity (g cm^{-3})	0.89	0.88	0.88	0.88	*0.04*
Density (kg cm^{-2})	3.10	2.90	3.00	3.00	*0.16*
Number of fruits on one plant (pcs)	6–18				
Number of commodity fruits on one plant (pcs)	9–12				

We found that the red tomato fruits of Iskorka variety had average diameter of 5.3 cm, weight of 93 g and average fruit volume of 105.5 cm^3. Six to 18 tomato fruits were harvested from one plant, 9–12 pcs correspond to standard (STN 3246–95, 1997).

Variation-and-statistical data processing in Table 7 indicated that the weather conditions in the year of tomato growing did not significantly affect the structural-and-mechanical properties of the fruits.

The development of the fruits occurred from the process of ovary formation to the end of growth. This stage was characterized by synthesis and accumulation of nutrients and intensive activities of metabolic processes (Overchenko, 2005).

Ripening of fruits causes increasing of the cells in size, weakening of intercellular adherence, intercellular spaces become wider, which leads to a change in the consistency of pulp, that is why their density decreases (Overchenko, 2005; Philipchuk, 2005). This changes the physical density of the fruit, which depends on the anatomical structure, the thickness of the fruit walls and the skin.

All these indicators affect the quality of the produce. Thus, the fruits of sweet pepper of technical degree of ripeness had physical solidity of 1.03 cm^3, which is almost 6% more than the fruits of biological degree of ripeness. Similar data were obtained in determining of mechanical strength of fruits – this indicator was reduced from 8.60 kg cm^{-2} in sweet pepper to technical degree of ripeness to 8.10 kg cm^{-2} of fruits of biological degree of ripeness, that was also by 6%. Consequently, fruits of sweet pepper of biological degree of ripeness had greater diameter and weight, but density and hardness were less. Most physical solidity does not worsen the quality of fruit, but obviously improve its transportation.

Peculiarities of the variety significantly influenced the density and hardness of the fruit. Physical density in eggplants of Helios variety was 1.13 g cm^{-3}, which was 14% less than in the fruits of Almaz variety. At the same time, hardness of the pulp of the last variety was 9% higher and was 7.90 kg cm^{-2}. Red tomato fruits of Iskorka variety had a tender consistency of the pulp and relatively low fruit density (0.88 g cm^{-3}) and mechanical strength (3.00 kg cm^{-2}).

Fruits for using in the processing industry often require the implementation of a number of technological operations, one of which is cleaning, which results in the removal of peduncle, seeds and pulp from the fruit. Waste share' indicator has certain economic and domestic value, since it determines the size of the commodity part of the fruit for different types of processing and consumption.

The ratio of separate parts of the fruit to the total weight of fruiting vegetables is given in Tables 8–10. We defined a significant varietal difference between the fruits of eggplant by the amount of inedible part of the fruit (seeds and peduncle). Thus, inedible part of the fruit in eggplant of Almaz variety, on average,was 14.3 g, which was about 10% of the fruit weight, while 13.07 g in Helios variety, which corresponded to only 6% of the fruit weight.

Table 8. Characteristics of separate parts of eggplant fruits

Part of fruit		Variety		LSD_{05}
		Almaz	Helios	
Pulp	(g)	110.20	178.73	*7.32*
	(%)	76.26	84.80	*4.04*
Skin	(g)	20.00	19.00	*0.98*
	(%)	13.84	9.00	*0.58*
Inedible part,	(g)	14.30	13.07	*0.70*
including:	(%)	9.90	6.20	*0.42*
seeds	(g)	4.43	4.05	*0.22*
	(%)	3.07	1.92	*0.13*
peduncle	(g)	9.87	9.02	*0.48*
	(%)	6.83	4.28	*0.29*

Moreover, the proportion of seeds-to-fruit weight was 4–4.5 g, which was about 2% in fruits of eggplant of Helios variety and 3% in the fruits of Almaz variety. The share of seeds in the eggplant fruits of both species under research was about 30% of inedible part.

It was found that the weight of the skin (edible part of the fruit) in eggplants of Almaz variety was 20 g or on average 14%, while it was 19 g in Helios variety, corresponding to only 9% of the fruit weight.

Eggplant fruits of Helios variety differed in hardness of pulp. Taking into account the ratio of the above-mentioned parts of the fruit, pulp in eggplant fruits of Helios variety was on average 85% and only 76% in Almaz variety.

Inedible part in sweet pepper includes seeds, seed cavity and peduncle and it is 15.2% in fruits of technical degree of ripeness and 18% in biological degree of ripeness which is 1.2 times more. At the same time, the share of seeds in the inedible part of the fruit of technical degree of ripeness was about 22%, and it was almost 4% more – 25% in biological degree of ripeness.

Table 9. Characteristics of separate parts of sweet pepper fruits of Novohohoshary variety

Part of fruit		Degree of ripeness		LSD_{05}
		technical	biological	
Pulp and skin	(g)	85.17	84.46	*4.22*
	(%)	84.75	82.00	*4.16*
Inedible part,	(g)	15.33	18.54	*0.86*
including:	(%)	15.25	18.00	*0.84*
seeds	(g)	3.32	4.70	*0.22*
	(%)	3.30	4.56	*0.20*
seed cavity	(g)	4.13	5.01	*0.23*
	(%)	4.11	4.86	*0.23*
peduncle	(g)	7.88	8.83	*0.43*
	(%)	7.84	8.58	*0.42*

Seed cavity in pepper was about 27%, regardless of the degree of ripeness. The seeds of sweet pepper fruits were ripe, and of milk or wax ripeness in technical degree. Pulp in sweet pepper fruits of technical and biological degree of ripeness was on average 82–85%.

The ratio of separate parts of the fruit to the total weight of tomato is presented in Table 10. Inedible part of the fruit in tomatoes is represented by seeds, the weight of which is 3–4 g per fruit, that is about 5% of the weight. Pulp and skin of tomato fruits are considered as edible parts, which in total occupy more than 88% of the fruit.

Variation-and-statistical data processing of Table 10 indicates that the weather conditions of the growing year did not significantly affect the number of separate parts of the tomato fruit of Iskorka variety.

Table 10. Characteristics of separate parts of tomatoes fruits of Iskorka variety

Part of fruit		Year				LSD_{05}
		2007	2008	2009	average	
Pulp and skin	(g)	88.03	88.62	88.40	88.35	*4.34*
	(%)	95.01	95.04	94.95	95.00	*4.80*
Inedible part, including:	(g)	4.62	4.63	4.70	4.65	*0.23*
	(%)	4.99	4.96	5.05	5.00	*0.24*
seeds	(g)	3.20	3.08	3.20	3.16	*0.16*
	(%)	3.45	3.30	3.44	3.40	*0.17*
peduncle	(g)	1.42	1.55	1.50	1.49	*0.08*
	(%)	1.53	1.66	1.61	1.60	*0.09*

CONCLUSIONS

Structural-and-mechanical indicators determine the suitability of fruiting vegetables for harvesting and preservation, as well as the ratio of separate parts of the fruit.

The established biometric parameters of the fruits and yield of tomatoes, sweet peppers and eggplants are aligned with the results of studies in other countries. However, foreign standards do not take into account commercial impact of the density of fruit and the amount of inedible fruit.

We have determined that the fruits of sweet pepper of technical ripeness are superior to the fruits of biological ripeness in terms of physical density and mechanical strength. Eggplant fruits of Almaz variety have higher values of fruit density than those of Helios variety. This will obviously have a positive impact on their transportation and delivery time.

The fruits of sweet biological ripeness have greater values of fruit length, width, weight and size than technically ripe fruits. Considerable varietal difference of eggplant fruits by the amount of edible and inedible parts of the fruit was determined. The inedible part (seeds and peduncle) in the eggplant fruits of Almaz variety was about 10% of the fruit weight, while it was only 6% in the eggplants of Helios variety. This is likely to be commercially viable.

REFERENCES

Bartolini, S. & Ducci, E. 2017. Quality evaluation of local apple varieties: physicochemical and antioxidant proprieties at harvest and after cold storage. *Agronomy Research* **15**(5), 1866–1877.

Darby, K., Batte, M.T., Ernst, S. & Roe, B. 2008. Decomposing local: A conjoint analysis of locally produced foods. *Am. J. Agr. Econ*. **90**(2), 476–486.

Corbo, M.R., Nobile, M.A.D. & Sinigaglia, M. 2006. A novel approach for calculation shelf life of minimally processed vegetables. *International Journal of Food Microbiology* **106**, 69–73.

Denver, S. & Jensen, J.D. 2014. Consumer preferences for organically and locally produced apples. *Food Qual. Prefer*. **31**, 129–134.

Freitas, M.A. & Costa, J.C. 2006. Shelf life determination using sensory evaluation scores: A general Weibull modeling approach. *Computers & Industrial Engineering* **51**, 652–670.

Leccese, A., Bartolini, S. & Viti, R. 2012.Genotype, harvest season, and cold storage influence on fruit quality and antioxidant properties of apricot. *Int. J. Food Prop*. **15** 4), 864–879.

Lima, V.L.A.G., Mélo, E.A., Maciel, M.I.S., Prazeres, F.G., Musser, R.S. & Lima, D.E.S. 2005. Total phenolic and carotenoid contents in acerola genotypes harvested at three ripening stages. *Food Chem* **90**, 565–568.

Osadcuks, V. & Pecka, A. 2016. Electrical field based detection of fruits and vegetables for robotized horticulture. *Agronomy Research* **14**(S1), 1088–1098.

Overchenko, B. 2005. Storage of fresh vegetables. *Vegetable production* **8**, 62–65 (in Ukrainian).

Philipchuk, P. 2005. Honor and praise orange! *Ogorodnik* **3**, 12 (in Ukrainian).

Rubatzky, V.E. & Yamaguchi, M. 1997. Word Vegetables: Principles, Production, and Nutritive Values. *2nd edition. Chapman & Hall, 115 Fifth Ave.* New York, NY. 843 pp.

STN 2660–94. Eggplant freshi. Specifications. Kyiv: Standartinform Publ., 14 pp. 1995.

STN 87561–79. Fruit and vegetable processing product. Methods for determining organoleptic quality assessment. Moscow: Standartinform Publ., pp. 1–12. 1979.

STN 2659–94. Sweet pepper fresh. Specifications. Kyiv: Standartinform Publ., 14 pp. 1995.

STN 3246–95. Tomatoes are fresh. Specifications. Kyiv: Standartinform Publ., 14 pp. 1997.

Yeshchenko, V.O., Kopytko, P.G., Opryshko, V.P., Kostogriz, P.V. 2005. *Fundamentals of Research in Agronomy*: textbook. Kyiv: Action, 288 pp. (in Ukrainian).

Agronomy Research **17**(6), 2295–2305, 2019
https://doi.org/10.15159/AR.19.195

Development of Latvian land use and land use change matrix using geospatial data of National forest inventory

L.L. Krumsteds*, J. Ivanovs, J. Jansons and A. Lazdins

Latvian State Forest Research Institute "Silava", Riga street 111, LV-2169 Salaspils, Latvia
*Correspondence: linards.krumsteds@silava.lv

Abstract. Land use and land use change calculation matrix is one of the most important parts of the national greenhouse gas (GHG) inventory in land use, land use change and forestry (LULUCF) sector providing information of an overall summary and changes in land use at a national level over a specified period of time. Information on land use and land use changes are further used to calculate other parameters important for determination of GHG emissions and carbon stock changes in living and dead biomass, soil and litter, as well as basic information on the impact of applied climate change mitigation measures. Calculations of land use change can be carried out in a partly automated process using GIS tools, which makes calculations easier to perform, reduces time consumption for this task and occasional mistakes due to manual operations. The aim of this study is to improve the methodology for development of land use and land use change matrix in the national GHG inventory system using geospatial data of National forest inventory (NFI) and auxiliary data sources. The developed system uses geospatial NFI data and auxiliary information provided by the land parcel information system (LPIS) and stand-wise forest inventory, and it improves accuracy and consistency of the land use and land use change matrix, providing the ability to apply the same land use accounting method for the whole reporting period since 1990 without a significant increase of uncertainty. The developed method determines land use changes in a 5-year period by comparing three successive NFI cycles. To determine the actual land use category in a particular year, we adjusted weights for different land use categories. Interpolation is used to determine year-by-year transitions.

Key words: Land use and land use changes, land use matrix, national forest inventory, greenhouse gas inventory.

INTRODUCTION

Land use and land use change as an interdisciplinary scientific topic has emerged only recently and the importance of it is also acknowledged by national and international research organizations. For instance, the United States of America National Research Council has identified it as a one of the seven grand challenges in environmental science (Brown et al., 2019). In land use, land use change, and forestry (LULUCF) land use values and results of the GHG emission estimates are the aftermath of a complicated intercommunication between social and ecological factors (Desta et al., 2000). Land use information provides knowledge on how society uses land resources, which is one of the key elements for accounting a projection of GHG emissions. Humans have modified land for their benefit and well-being, throughout history they have performed activities like cropping, grazing, logging, mining and urbanization and these processes are still in action at present time (Sleeter et al., 2012; Holman et al., 2017; Wulder et al., 2018). Human material, social, and cultural needs have been and still are provided by the land and its resources (Mekkonen et al., 2018; Birhane et al., 2019). However, nature still plays a role in land use changes, which can cause either a positive or a negative impact (Gomes et al., 2019).

The main reason why land use and cover have caught global academic and political attention is its standpoint as a primary factor characterizing direct influence to ecosystems and the factor that responds in different ways, depending from land use, on global climate change (Cegielska et al., 2018; Hersperger et al., 2018). One of the monitoring tools used for land use and land use change is National forest inventory. It is an important tool to construct historical and long term monitoring system, which can provide data about land use and land use changes (Soulard & Wilson, 2013). In LULUCF sector land use is divided into 6 main categories – forest land, cropland, grassland, wetlands, settlements and other lands. These land use categories according to Intergovernmental Panel on Climate Change (IPCC) guidelines are split into 2 groups – areas, where land use change took place 20 years ago or more recent and other lands, where land use changed more than 20 years ago or no land use change took place during the accounting period (Eggleston et al., 2006).

National forest inventory (NFI) has been introduced in most of the European countries because of the need for a national and regional sample based multi-resource forest inventories (Traub et al., 2017). Perpetual monitoring programs, like national forest inventories, are a significant source of information for ecological and environmental research and decision making (Lindenmayer & Likens, 2010). NFI is a part of Latvia’s forest monitoring program and it produces estimates of numerous parameters describing the current status and changes of forest resources – information needed for policy and decision making at national and subnational levels, as well as for international reporting (Pulkkinen et al., 2018). National forest inventory in Latvia is implemented since 2004 by Latvian State Forest Research Institute Silava (LSFRI Silava), which is appointed by the national responsible authority – the Ministry of Agriculture. Each year before April 1st LSFRI Silava submits the information obtained during the previous year’s NFI to the Ministry of Agriculture.

Since 2008 LSFRI Silava is responsible for data collection necessary for reporting of carbon stock changes and GHG emissions, and NFI data is the main source of activity data, which is used in the national GHG inventory (Jansons & Licite, 2010). The methodology currently used for accounting of carbon stock changes is listed as the most comprehensive approach – the utilized methods can be applied in a similar way for any type of land use (i.e., generic methods for Forest Lands, Croplands, Grasslands, Wetlands, Settlements and Other land) (Eggleston et al., 2006).

The main goal of this study is to develop and improve land use and land use change matrix in the national GHG inventory system using geospatial data from NFI and auxiliary data. Geographical information systems (GIS) are used in this study because the IPCC guidelines require accounting of land use and land use changes in a spatially explicit way, therefore GIS tools is the only reliable tool to process land use and land use change data. GIS tools can relatively easy store, capture and analyze geospatial data without intermediate solutions, which is important when implementing study results into practice (Clarke et al., 2019). Automatization of this process using GIS tools is aimed at improving the speed of data processing because the new calculation method will considerably reduce the probability of mistakes, demand for expert judgements and time consuming manual data sorting that was necessary prior to this study to develop the land use and land use change matrix.

MATERIALS AND METHODS

Study area

NFI plots are scattered through the whole territory of Latvia, in total there are 16,156 permanent plots (Fig. 1). Every plot represents an area of 400 ha and each plot is measured once during the 5-year period. Sample plots can be divided into smaller units called sectors, which are created, if the plot is situated on a boundary of different land use categories or vegetation types (Jansons & Licite, 2010). Each compartment, also called sector, contains information about land use category and stand inventory information, if there are trees in the specified sector (Fig. 5).

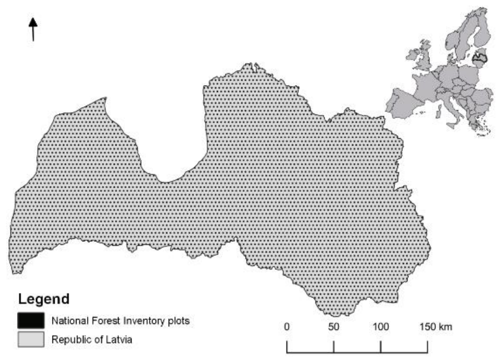


Figure 1. Study area.

Geospatial data processing

One of the goals of the study is to create a calculation method that takes into account possible land use category changes through 3 NFI cycles (15 years) and permanent land use change categories (properties of these land use change categories have permanent or long-lasting possibility, such as forest roads, railway tracks, water bodies, etc.).

Three 5-year periods are intersected with each other, starting with the oldest one using GIS tools. In order to calculate final land use categories in a way that takes into account short time changes between NFI cycles, weights are added to the subsequent cycles as well as to categories that are unlikely to change easily (roads, water bodies, settlements, etc.). All land use and land use change categories with constant and non-changing properties are supplemented with weight value 1. Categories of three subsequent NFI cycles are supplemented with weighted values, which differ from their age. The oldest is supplemented with weight 20, the middle one - with weight 30, and the youngest one - with weight 50. Fields that meet the constant, non-changing properties will always be one value higher than those, who don't have it. Example: the oldest one will weigh 21, middle one 31, but oldest one 51. Weighted values then are used to calculate the land use in a particular period or year (Fig. 2). This process is repeated to all subsequent NFI cycles and previously obtained data, starting from the year 1985. For all previously mentioned processes we use GIS software and file format that supports curved line shape.

Fig. 2. shows the same NFI permanent plot through 3 cycles or 15 years. In spite of cycle 3 land use changes to grassland, the final category is defined as cropland. The reason for this estimate is the specific properties of calculation formula which indicate that the third cycle is not 'heavier' than the two previous cycles together, if the transition is not marked as non-reversible (construction, drainage ditch, etc.). Although the cycle 3 is closer to the present times and more relevant to the possible present-day situation, in this case, it is possible that grassland is only a temporal land use in this plot and in the future, it will turn back into cropland. If grassland in this plot will be detected also in the fourth cycle, the final land use category will be changed to grassland according to the logic of the calculation, and previously reported land use data will be recalculated, assuming that the land use change took place in the time between site visits in cycle 2 and 3.

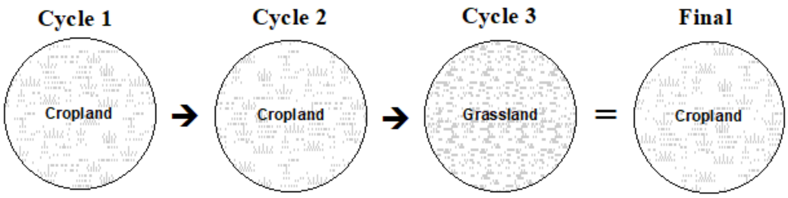


Figure 2. Example of evaluation of land use and land use change.

Fig. 3 shows a scenario, where cropland in the cycle 2 changes into cropland but because land use category in cycle 1 and cycle 3 is grassland, the final land use category remains grassland. This scenario represents situations, when calculation method takes into account that land use category can change periodically for a short period of time. In some cases, land owners change their land use to cropland for a period of one year to meet personal land management needs, thus influencing land use change information for the specific information gathering cycle and immensely impacting information on land use and land use change in the long run. This occurs because of the specific field data gathering method, which dictates that each individual plot is monitored only once in a 5-year period also called cycle and the gathered information will represent land use in the specific plot for the whole cycle.

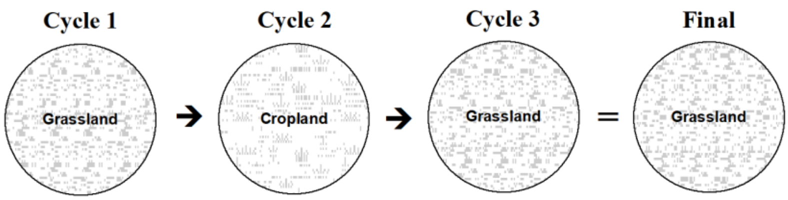


Figure 3. Example of evaluation of land use and land use change.

Fig. 4. represents a scenario, when land use type in the two older cycles is forest land but in the newest cycle the settlement category appears across the plot, which indicates that forest road construction has taken place recently, thus changing the land use category to settlement in cycle 3 and also influences calculated final land use category, which adjusts accordingly to the new changes, because forest roads have non-

reversible land use property. Non-reversible land use property indicates that it cannot change easily in a long period of time. Buildings, roads, drainage ditches and other structures of anthropogenic origin, which are components of the settlement category, leave a long lasting impact on land use change process because their longevity comparing to different land use category ingredients is far greater, the only exceptions are lakes and rivers which fall into the wetland category and also have non-reversible land use property. Non-reversible land use property also protects from possible mistakes in land use category classification during field works in future, because if by any chance non-reversible ingredients are not detected in future cycle field works it will be represented in calculated final land use category, thus making it one of the data processing safety mechanisms.

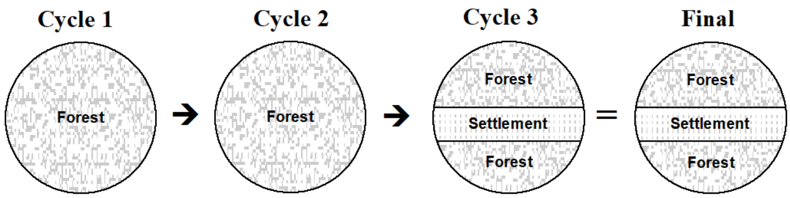


Figure 4. Example of evaluation of land use and land use change.

Fig. 5 illustrates how differently weighted categories interact with each other to transfer to the final land use category. If the interaction is between categories with different weights (regarding land use category), the final land use category will be the same as the category that has the 'heaviest' value (for example, orchard).

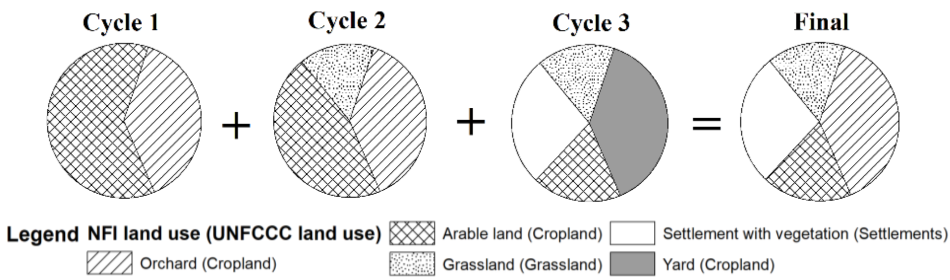


Figure 5. Illustration of land use and land use change.

If interaction between the three cycles is between categories with the same weight of land use categories, then the final land use category will be the category that is repeated at least 2 times, or in case, if the land use category is different in each cycle, the latest category will be applied as the final land use category. The reason for this is the fairly rapid changes of land use in the particular NFI plots, which can be caused by human error. To avoid previously mentioned problems in reporting of the land use and land use changes, we propose a method that considers the previous three NFI cycles for deciding the final land use category.

Land use and land use change matrix

Land use and land use change matrix is calculated using the final land use category, which is estimated by combining 3 subsequent NFI cycles. NFI plot areas are calculated in such a way so that altogether they represent the total area of Latvia. The first step is to calculate the proportion between the area of all the NFI plots and the area of Latvia,

$$\text{Unit coefficient} = \frac{\sum \textit{of all plot shape area}}{\textit{Country area}} \tag{1}$$

and the next step – to calculate the representative area of each part of the NFI plot,

$$\text{NFI plot area in ha} = \frac{\sum \textit{of all the plot shape area}}{\textit{Unit coefficient}} \tag{2}$$

The layer, which represents the final land use category, is then intersected with a polygon layer, which represents areas of permanent grasslands, which are obtained from the LPIS, maintained by the Rural Support Service. This step is necessary because of the complexity of evaluation of the situation in the field during the site visit by the NFI teams. When data collection is conducted by the specialists in the field, it cannot be precisely determined if grassland is natural or it is manually sown and if it is periodically plowed. Data provided by the Rural Support Service helps to eliminate potential errors during the field works. If the land use category in the final land use category layer is representing grassland and plot overlaps with cultivated grassland polygon, the land use category is changed to cropland.

While the Latvian NFI in total has 49 land use categories, the UNFCCC has only 6 land use categories. Land use and land use change categories that are consolidated from the NFI database are specially made for the NFI purposes, but they are easily transformable to the UNFCCC categories - there is already a table available for conversion purposes. The result is the land use and land use change matrix that gives the values of area change between two different NFI cycles.

In total six land use change matrices were generated, each covering a 5-year cycle (Fig. 6). For the period of time before the NFI was started in Latvia in 2004, Landsat data was used. The first trials of Landsat data use for LULUCF needs in Latvia started in 2011 (Lazdins, 2011). Combined matrix, covering a time span from 1990 was also created at this time, however, it was also concluded that unguided classification of land use may lead to considerable uncertainty, particularly, considering the small size of the NFI plots in comparison to the spatial resolution of the Landsat data.

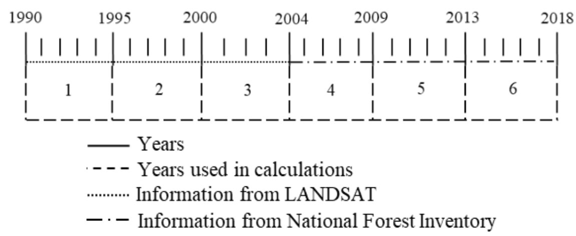


Figure 6. Years with information on land use and land use change used in elaboration of the land use change matrix.

Land use and land use change matrix - comparison between cycles by years

Comparison between matrices of different years is made by creating a template for all years of the reporting period, in this case, 1990–1995. Each land use category is compared with the other five land use categories to determine the area that has transferred from other land use categories each year. Constant values are obtained from previously calculated data matrices, which serve as ‘anchor’ values that are real and reliable. Other values that are between the ‘anchor’ values are calculated using linear interpolation.

$$\text{Last year area} = \frac{youngest\ cycle\ area - oldest\ cycle\ area}{\sum of\ years\ between\ youngest\ and\ oldest\ cycle} \quad (3)$$

Modifications for calculations of land use and land use change matrices in future

NFI field specialists monitor around 1/5 of all plots every year and only a partial land use category update is available. The necessity for yearly land use and land use change updates led to development of a modified calculation method that provides reliable data about land use changes for the last 10 years, which can be used further to calculate GHG emissions in the LULUCF sector. Every year we can add partial information to the database, where the newest accessible data replaces the oldest data (Fig. 7).

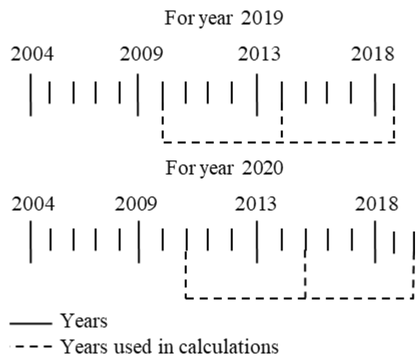


Figure 7. Yearly update of NFI database with partial information.

RESULTS AND DISCUSSION

Matrices can be created between each cycle or through all cycles. Table 1 shows land use and land use changes through all the cycles from 1990 to 2018 and describes, which land use category has gained area from other land use categories. Since 1990 the most noticeable change in land use in Latvia is the transformation of cropland to grassland and transformation of grassland to forest land (Table 1). It was caused by widespread abandonment of rural areas in Latvia and other post-soviet countries in the early nineties after the collapse of the Soviet Union (Prishchepov et al., 2012; Alcantara et al., 2013). That led to active afforestation and natural succession in the Baltic States and has resulted in an increase of forest land area (Lazdiņš et al., 2010), which is also observed in the Nordic region (Gundersen et al., 2014). A need for land use and land use change data have led to other studies, which have been conducted in Latvia to estimate land use and land use change data and trends. These studies have shown a decreasing trend for agricultural land and grassland and an increasing trend for forest land after the collapse of the Soviet Union in 1990 and these trends were the result of agricultural land abandonment, which led to increase in grassland and forest land areas resulting from ecological succession (Baders et al., 2018).

The graph in Fig. 8 represents all land use categories and land use changes each year since 1990. It needs to be taken into account that the wetland category also includes active and abandoned peat extractions fields, which were determined earlier using wall to wall approach. In 2016, when the total area of wetlands was around 404 kha, active and abandoned peat extraction fields constituted 34.2 kha (Butlers & Ivanovs, 2018). The only constant value that has not changed since 1990 is other lands. Other lands have not changed because the only land use category in NFI that can be reclassified to other lands is sandy dunes, which are located in the NFI plots nearby the Baltic sea. Values shown in Table 1 are the same as the ones in Fig. 8. The difference between Fig. 8 and Table 1 is that the Fig. 8 represents land use changes for each year separately, but Table 1 represents land use changes through the whole calculation period from 1990 to 2018.

Table 1. Land use and land use change matrix between the years 1990–2018 (all the matrices mentioned in this article and the complete in-depth set of matrices are available digitally at https://goo.gl/EgVHcx)

Land use change		Land use Land use at the end of the period						Sum before
		Settlement	Cropland	Other land	Forest land	Wetland	Grassland	
Land use at the start of the period	Settlements	263,115	3,072		19,807	1,131	5,352	292,476
	Cropland	16,346	1,399,117		56,150	10,780	578,809	2,061,201
	Other lands			5,437				5,437
	Forest land	21,507	3,559		3,091,588	22,505	38,161	3,177,319
	Wetlands	1,079	1,364		9,445	357,976	5,345	375,209
	Grassland	5,958	62,768		65,266	11,698	401,617	547,308
Sum after		308,004	1,469,880	5,437	3,242,255	404,090	1,029,284	6,458,950

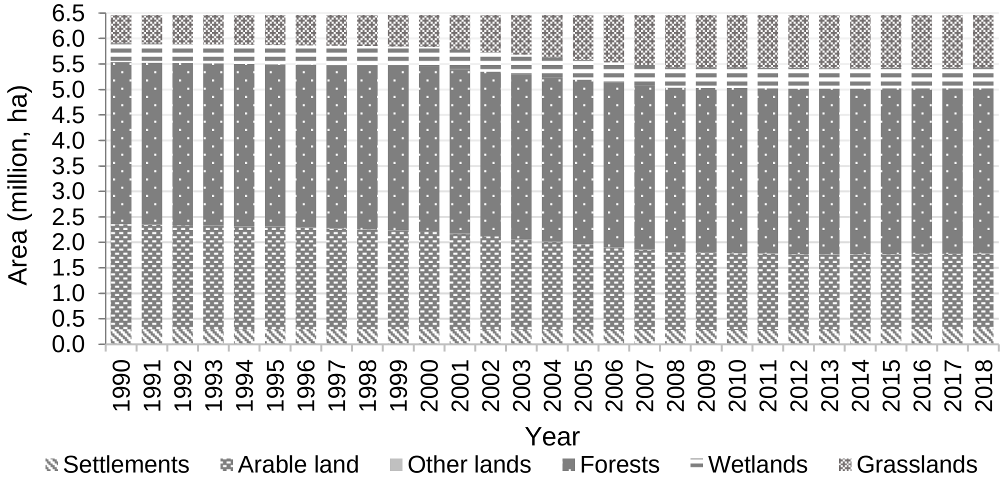


Figure 8. Land use and land use changes since 1990.

According to the study done by Baders et al. (2018), the proportions of land use categories in Latvia in the year 1990 was the following – 49.2% - forest lands, 26.3% - grasslands and 11.5% - croplands. Comparing with the results obtained in our study there are similarities in forest land category, but differences in cropland and grassland land use categories. Our study show that forest lands occupy 49.2% of the territory of Latvia, but grasslands occupy 8.5% and croplands 31.9%, indicating that the results of our study differ from the previously mentioned study accordingly: -17.8% for grasslands and +20.4% for croplands. The same study indicates that proportions of land use categories in 2011 in Latvia was: forest lands - 50.3%, grasslands - 23.7% and croplands - 12.7%, which is similar to results obtained in our study that shows the following proportions of land use categories in 2011: 50.2% - forest lands, 16% - grasslands and 23.1% - croplands. Difference from our studies in forest land is -0.1%, in grasslands -7.7% and in croplands

+10.4%. These differences indicate that the results of our study are impacted by the new calculation system, which takes into account probable land use changes throughout years, like periodical yearly changes in grassland and cropland categories, where one land use category can change into another by owners adapting the land use of their private property to their management needs and plans. Examples are shown in Fig. 2 and Fig. 3.

Individual land use change matrices for each land use category through the years also have been elaborated. Through the years it is possible to trace land use changes between different land use categories (Table 2). In this case, it is shown how cropland from 2013 to 2018 gains land area from other land use categories. This is only a part of the full size matrix which starts from the year 1990. A complete set of matrices is available digitally at https://goo.gl/EgVHcx. Even though values between the anchor years, which are coloured gray in the table, are calculated using linear interpolation, it gives a representational value to data and gives at least theoretical estimates on how land use has changed during the reporting period. Anchor values have been obtained from land use and land use change matrices, which were calculated previously.

Table 2. Land use change matrix between cropland and other land use categories from 2013 to 2018

Land use after	Land use before	2013	2014	2015	2016	2017	2018
Cropland	Settlements	2,502	2,090	1,678	1,266	854	443
	Cropland	1,437,871	1,441,609	1,445,348	1,449,086	1,452,824	1,456,563
	Other land	0	0	0	0	0	0
	Forest land	2,377	2,284	2,191	2,098	2,005	1,912
	Wetlands	1,364	1,251	1,138	1,025	912	800
	Grassland	27,355	23,917	20,478	17,040	13,601	10,163
	Total (ha)	1,471,469	1,471,151	1,470,833	1,470,515	1,470,198	1,469,880

The stand-wise forest inventory database of the State Forest Register (SFR) has been used to make alterations to previously generated land use and land use change data. It is assumed that the area, which is legally transferred into forest land, respectively, included in the SFR database, should be accounted as land converted to forest land. NFI plots and sectors that are accounted by NFI teams as forests on farmland or overgrown areas (NFI categories 62 and 64), and according to the NFI data are afforested after 1989 and are intersecting with the SFR database layer are transferred to land converted to forest land category. After those alterations additional 70’243 ha have been added to the category of afforested lands, in total accounting for 382’386 ha of land area.

The calculated land use and land use change data values are compared to the data reported for LULUCF and it shows similarities in land use and land use changes. The biggest observed differences are in cropland and grassland land use, where the calculated data have a 2.1–7.3% shift, comparing with the LULUCF data (Table 3). Those differences

Table 3. Relative differences between land use data according to the National Forest Inventory and land use data previously reported for LULUCF, (%)

	1990	1995	2000	2008	2013	2018
Settlements	+0.8	+0.8	+0.8	+0.6	+0.7	+0.9
Cropland	+3.5	+3.3	+2.1	-4.6	-6.3	-7.3
Other lands	0.0	0.0	0.0	0.0	0.0	0.0
Forest land	+0.8	+0.7	+0.8	+0.8	+0.9	+0.8
Wetlands	-1.1	-1.1	-1.1	-1.0	-0.9	-0.9
Grassland	-4.1	-3.7	-2.6	+4.2	+5.6	+6.5

have occurred because of the specific calculation method applied in land use and land use change calculations using NFI data, which is explained in Fig. 2 and Fig. 5, and auxiliary data usage like LPIS.

CONCLUSION

The estimated land use and trends in land use changes are similar to previously available land use and land use change data from LULUCF reports ensuring that the developed method is comparable with other data sources and can be used for land use and land use change calculations in future.

The proposed method considerably improves the quality of the activity data for GHG accounting in LULUCF sector by reducing non-existing land use changes like conversion of cropland to grassland and vice versa, by linearization of the trends of land use changes and by the implementation of recently available NFI data.

The elaborated GIS and linked spreadsheet tools have reduced the necessary time for land use calculations and also eliminated possible errors that might have occurred during manual calculations of activity data for land use and land use changes in Latvia.

Even though the obtained results are representable and meet the required demands, the use of auxiliary data like LPIS data is still recommended to eliminate possible impurities in the NFI data in reporting of impact of management activities and rare land use categories, e.g. peat extraction sites.

REFERENCES

Alcantra, C., Kuemmerle, T., Baumann, M., Bragina, E.V., Griffiths, P., Hostert, P., Knorn, J., Muller, D., Prishchepov, A.V., Schierhorn, F., Sieber, A. & Radeloff, V.C. 2013. Mapping the extent of abandoned farmland in Central and Eastern Europe using MODIS time series satellite data. *Environmental Research Letters* **8**(3), 9 pp.

Baders, E., Lukins, M., Zarins, J., Krisans, O., Jansons, A. & Jansons, J. 2018. Recent land cover changes in Latvia. *Annual 24th International Scientific Conference Research for Rural development 2018.* **1**, pp. 34–39.

Birhane, E., Ashfare, H., Fenta, A.A., Hishe, H., Gebremedhin, M.A., Wahed, H.G. & Solomon, N. 2019. Land use land cover changes along topographic gradients in Hugumburda national forest priority area, Northern Ethiopia, Remote Sensing. *Society and Environment* **13**, 61–68.

Brown, J.F., Tollerud, H.J., Barber, C.P., Zhou, Q., Dwyer, J.L., Vogelmann, J.E., … Rover, J. 2019. Lessons learned implementing an operational continuous United States national land change monitoring capability: The Land Change Monitoring, Assessment, and Projection (LCMAP) approach. *Remote Sensing of Environment.* In review process.

Butlers, A. & Ivanovs, J. 2018. Improved activity data for accounting greenhouse gas emissions due to management of wetlands. *Annual 24th International Scientific Conference Research for Rural Development 2018.* **1**, pp. 27–33.

Cegielska, K., Noszczyk, T., Kukulska, A., Szylar, M., Hernik, J., Dixon-Gough, R., … Filepné Kovács, K. 2018. Land use and land cover changes in post-socialist countries: Some observations from Hungary and Poland. *Land Use Policy* **78**, 1–18.

Clarke, R., Sosa, A. & Murphy, F. 2019. Spatial and life cycle assessment of bioenergy-driven land-use changes in Ireland. *Science of the Total Environment* **664**, 262–275.

Desta, L., Kassie, M., Benin, S. & Pender, J. 2000. *Land degradation and strategies for sustainable development in the Ethiopian highlands: Amhara Region*. Socio-economics and Policy Research Working Paper 32. ILRI (International Livestock Research Institute), Nairobi, Kenya, 122 pp.

Eggleston, H.S., Buendia, L., Miwa, K., Ngara, T. & Tanabe, K. (eds). 2006. *IPCC Guidelines for National Greenhouse Gas Inventories, Prepared by the National Greenhouse Gas Inventories Programme.* Volume **4**. *Agriculture, Forestry and Other Land use.* IGES, Japan.

Gomes, E., Abrantes, P., Banos, A., Rocha, J. & Buxton, M. 2019. Farming under urbanpressure: Farmers' land use and land cover change intentions. *Applied Geography* **102**, 58–70.

Gundersen, P. (Ed.), Ginzburg Ozeri, S., Vesterdal, L., Bárcena, T.G., Sigurdsson, B.D., Stefansdottir, H.M., ... Lazdina, D. 2014. *Forest soil carbon sink in the Nordic region.* Frederiksberg: Department of Geosciences and Natural Resource Management, University of Copenhagen. IGN Report.

Hersperger, A.M., Oliveira, E., Pagliarin, S., Palka, G., Verburg, P., Bolliger, J., Grădinaru, S. 2018. Urban land-use change: The role of strategic spatial planning. *Global Environmental Change* **51**, 32–42.

Holman, I.P., Brown, C., Janes, V. & Sandars, D. 2017. Can we be certain about future land use change in Europe? A multi-scenario, integrated-assessment analysis. *Agricultural Systems* **151**, 126–135.

Jansons, J. & Licite, I. 2010. Chapter 21, Latvia. In Tomppo, E., Gschwantner, Th., Lawrence, M., McRoberts, R.E. (Eds.), *National Forest Inventories. Pathways for Common Reporting.* Springer, London, pp. 341–350.

Lazdins, A. 2011. Harmonization of land use matrix in Latvia according to requirements of international greenhouse gas reporting system - extending outputs of National Forest inventory program. *Collection of Abstracts.* Latvia University of Agriculture, Faculty of Social Sciences, Faculty of Engineering, Forest Faculty, Jelgava, 10.

Lazdiņš, A., Lazdiņa, D. & Liepa, I. 2010. Characterization of naturally afforested farmlands in Latvia. In *Annual 16th International Scientific Conference Proceedings* (Vol. **1**, pp. 176–183). Jelgava: Latvia University of Agriculture.

Lindenmayer, D.B. & Likens, G.E. 2010. The science and application of ecologicalmonitoring. *Biological Conservation* **143**(6), 1317–1328.

Mekonnen, Z., Berie, H.T., Woldeamanuel, T., Asfaw, Z. & Kassa, H. 2018. Land use and landcover changes and the link to land degradation in Arsi Negele district, CentralRift Valley, Ethiopia, Remote Sensing. *Society and Environment* **12**, 1–9.

Prishchepov, A.V., Radeloff, V.C., Baumann, M., Kuemmerle, T. & Muller, D. 2012. *Environmental Research Letters* **7**(2), 13 pp.

Pulkkinen, M., Ginzler, C., Traub, B.& Lanz, A. 2018. Stereo-imagery-based post-stratification by regression-tree modeling in Swiss National Forest Inventory.*Remote Sensing of Environment* **213**, 182–194.

Sleeter, B.M., Sohl, T.L., Bouchard, M.A., Reker, R.R., Soulard, C.E., Acevedo, W., … Zhu, Z. 2012. Scenarios of land use and land cover change in the conterminous United States: Utilizing the special report on emission scenarios at ecoregional scales. *Global Environmental Change* **22**(4), 896–914.

Soulard, C.E. & Wilson, T. 2013. Recent land-use/land-cover change in the Central California Valley. *Journal of Land Use Science* **10**(1), 59–80.

Traub, B., Meile, R., Speich, S. & Rösler, E. 2017. The data storage and analysis system of the Swiss National Forest Inventory. *Computers and Electronics in Agriculture* **132**, 97–107.

Wulder, M.A., Coops, N.C., Roy, D.P., White, J.C. & Hermosilla, T. 2018. Land cover 2.0. *International Journal of Remote Sensing* **39**(12), 4254–4284.

Agronomy Research **17**(6), 2306–2316, 2019
https://doi.org/10.15159/AR.19.211

Determination of activation energy of the pellets and sawdust using thermal analysis

D. Kunecová and P. Hlaváč

Slovak University of Agriculture in Nitra, Faculty of Engineering, Tr. A. Hlinku 2, SK-949 76 Nitra, Slovak Republic
Correspondence: kunecova@uniag.sk; peter.hlavac@uniag.sk

Abstract. The aim of this study is to describe the thermophysical properties of pellets and sawdust. Samples were chosen with regard to sustainability and environmental friendliness of materials. The main object of this paper was the investigation of thermal degradation of selected samples. Industrial pellets from Slovakia and sawdust from household source were compared. Materials suitable for pyrolysis are organic materials that degrade at increased temperature. Cellulose, hemicellulose and lignin are main components of biomass (wood) in varying proportions. Thermal processes were carried out from 25 °C to 850 °C using inert nitrogen atmosphere. Heating rate was linear from 5 °C min^{-1}, 10 °C min^{-1} to 20 °C min^{-1}. Mass decrease to 150 °C corresponds to release of water and other lighter unbound hydrocarbons. Samples lose 6–8% of their mass due to the temperature. At main decrease the mass loss was between 62% and 69%. In some cases this decrease is in two drops that end at the temperature around 500 °C. Pyrolysis can be considered to consist of independent parallel reactions. In order to make theoretical groundwork for biomass pyrolysis available, activation energies were calculated with the help of two kinetic models (Kissinger-Akahira-Sunose and Flynn-Wall-Ozawa model). The residuals from pyrolysed samples are determined and temperature dependent profiles of the materials were obtained.

Key words: activation energy, biomass-waste, pyrolysis, thermogravimetric analysis.

NOMENCLATURE:

DTG – Differential Thermogravimetric Analysis
FWO – Flynn-Wall-Ozawa
KAS – Kissinger-Akahira-Sunose
TGA – Thermogravimetric Analysis
A (s^{-1}) – Pre-exponential factor
E_a (kJ mol^{-1}) – Activation energy
R (8.314 J mol^{-1} K^{-1}) – Universal gas constant
T (K) – Temperature
$T_{\alpha i}$ (K) – Temperature at conversion i
m (kg) – Mass

Subscripts
T – Instantaneous mass
0 – Initial mass
k – Final mass

Greek symbols
α (–) – Conversion
$\frac{d\alpha}{dt}$ (s^{-1}) – Conversion rate
β (K.min^{-1}) – Heating rate.

INTRODUCTION

The aim of this study is to describe thermophysical properties of pellets from wood and wood chips. Biomass is an organic product generated by a natural process. It is used and consumed in human activities. Biomass waste is currently adequately utilized in order to meet the energy demands of the population. Since the past decades, reserves of fossil fuels which are the major energy source in most countries are continuously being depleted (Blaschke et al., 2013; Jouhara et al., 2018). It is therefore favourable to seek replacement of these resources in more accessible commodities. Biomass can be this resource, for its availability and production amount. Natural biomass, special wood waste, is a renewable energy source, while biomass fuel is a sustainable energy resource (Suriapparao et al., 2014; Suzdalenko et al., 2014; Tomei & Helliwell, 2016). Since it is recognised that the biomass system and respective biofuels as sub-systems do not contribute to the greenhouse effect (due to the CO_2 neutral conversion), extensive investigations have been carried out worldwide to enhance the biomass use by substituting fossil fuels for energy conversion (Vassilev et al., 2010). Energy from the biomass can be obtained by thermal decomposition without access of air, reduced oxidant feed or simple burning. The thermal method of thermogravimetric analysis resembles the pyrolysis. Pyrolysis is the thermal degradation of substance at elevated temperature in the absence of oxygen or in a reduced oxygen atmosphere. Pyrolysis and gasification are the most commonly employed and the most appropriate processes for the generation of liquid biofuels, syngas, chemicals, or charcoal via pyrolysis and liquefaction processes (Soria-Verdugo et al., 2013; Chan et al., 2015). Most of the biomass has heterogeneous property attributes due to the fact that the biomass itself constitutes of numerous components, such as hemicellulose, cellulose, lignin, and minor amounts of extractives (Hu et al., 2017). These differences in biomass composition determine the nature of thermal decomposition and individual decomposition processes. Thermogravimetric analysis allows us to look at the thermal behaviour of biomass under various conditions of the oxidation environment (Nilova et al., 2017; Tamelová et al., 2019). We can also determine the shape of the thermogravimetric curve and thus the individual decomposition temperatures of the biomass components. According to the shape of the TGA curves, the pyrolysis of biomass materials determine the rate of the reaction and the temperature of the decomposition processes. From thermal degradation of materials we can identify thermal resistance, rate of processes, temperature of degradation and activation energy. The non-isothermal methods are the most commonly used for performing the kinetic analysis of solid state reactions (Fernandez et al., 2018). The kinetic analysis is conducted using an independent parallel reaction model to extract non-isothermal pyrolysis kinetic parameters including activation energy in the feedstocks. An independent parallel reaction model is adopted for modelling to ensure that the pseudo-components are degraded simultaneously and individually in a same temperature range (Bach et al., 2019). Interactions among the biomass pseudo-components are neglected. Individual pseudo-components can be described by the Eq. 1 (Ali et al., 2017; Bartocci et al., 2019). By using the TGA data, the kinetic parameters as well as pyrolysis mechanism can be determined according to different mathematical approaches (Hu et al., 2017). Pyrolysis has been employed to convert biomass feedstock into bio-fuels with high energy density, and biomass pyrolysis generates three product streams: liquid (bio-oil), gas, and solid product (biochar) (Liu & Han, 2015). The distributions and properties of these product streams are strongly dependent on pyrolysis conditions (Zhengang & Guanghua, 2015). Wood is a composite material consisting of cellulose, hemicellulose and lignin as major cell wall constituent polymers with small amounts of minor components, including inorganic substances. Lignin is hydrophobic and is found to be covalently linked to the structures of hemicellulose (Hosoya et al., 2007; Rana et al., 2018). Furthermore, vapour phase carbonization of the volatile products from lignin was substantially inhibited in wood and demineralized wood. Any material containing major components of biomass, such as lignin, cellulose and hemicellulose, decomposes at 200 °C. This main decomposition proceeds to the temperature of 600 °C, where cellulose, lignin and hemicellulose are degraded. Despite this main decomposition, all mass changes during the course of degradation are important for the determination of kinetic parameters. By examining the kinetic parameters, we can determine the activation energy of the material in special conditions depending on the size of the conversion. The activation energy can be explained as energy that is required to activate atoms and molecules to a condition in which they can undergo chemical transformation or physical transport (Hlaváč et al., 2016).

MATERIALS AND METHODS

Thermogravimetric analysis was performed on two samples from different sources using the Mettler Toledo TGA/DSC 1 instrument. Samples were chosen with regard to sustainability and environmental friendliness of materials. Industrial pellets from Slovakia and waste wood from household source were compared. Pellets and waste wood were not specifically modified. Samples were provided by Slovak company for pellets production. High quality pellets from mix of soft woods like spruce with low ash content, around 0.80%. Waste wood was sawdust and it arose by treatment of wood in household, predominantly spruce. Samples were weighted on KERN 220-5M scales with an accuracy of 0.01 mg to 0.1 mg. Graphs were created in STAR^{e} SW 12.10 software and kinetic parameters were calculated by using Excel.

TGA was performed at three different heating rates: 5 °C min^{-1}, 10 °C min^{-1} and 20 °C min^{-1}. Masses of samples were in the range 92–101 mg for pellets and 23–30 mg for sawdust and were placed in a closed aluminia container. The temperature program was set from 25 °C (room temperature) to 850 °C, at a nitrogen atmosphere with a flow rate of 50 mL min^{-1}. Measurements were executed an inert environment without access of air.

Kinetic parameters of biomass thermal degradation can be calculated by Arhenius law

$$\frac{d\alpha}{dt} = Ae^{\left(-\frac{E_a}{RT}\right)}f(\alpha) \tag{1}$$

where α – conversion; R – universal gas constant; A – pre-exponencial factor; T – thermodynamic temperature; E_a – activation energy and f(α) – reaction model, J mol^{-1} K^{-1}; K; kJ mol^{-1}. Value of the conversion or extent of the conversion during pyrolysis can be expressed as

$$\alpha = \frac{m_0 - m_t}{m_0 - m_k} \tag{2}$$

where m_0 – initial mass, mg; m_t – immediate mass (mg) and m_k – final mass, mg; (Vyazovkin et al., 2011; Ondro et al., 2017). At a constant heating rate (β) the temperature changes are linear with time, and the iso-conversional methods can be used for the determination of kinetics parameters. The iso-conversional methods require performing a series of experiments at different temperature programs and yield the values of effective activation energy as a function of conversion. More often the activation energy is found to vary with the extent of conversion. Iso-conversional methods are widely used in literature for evaluation of the activation energy.

KAS model (Kissinger-Akahira-Sunose) is one of the best methods, used for the relation between heating rate and inverse temperature, by relationship

$$\ln\left(\frac{\beta_i}{T_{\alpha i}^2}\right) = const - \frac{E_\alpha}{RT_{\alpha i}} \quad (3)$$

The activation energy is given as the slope of linear fit of function of natural logarithm $\frac{\beta_i}{T_{\alpha i}^2}$ and inverted value of the temperature (Starkin et al., 2003; Vyazovkin & Sbirrazzuoli, 2006; Damartizs et al., 2011; Ali et al., 2017).

FWO model (Flynn-Wall-Ozawa) is one of the most commonly accepted methods for the computation of kinetics parameters. The method uses data from thermogravimetric analysis for the determination of the activation energy. The activation energy is obtained from the slope of the linear fit of the plot between ln(β_i) and inverted value of the temperature. The integral solution after integration with respect to temperature is shown by Eq. (4)

$$\ln \beta_i = const - 1.052\left(\frac{E_\alpha}{RT_\alpha}\right) \quad (4)$$

(Flynn & Wall, 1966; Vyazovkin & Sbirrazzuoli., 2006; Damartizs et al., 2011; Ali et al., 2017).

RESULTS AND DISCUSSION

When comparing individual measurements, only the expected change in the same sample and different heating rates was evident. As the heating rate increases, the TGA curve shifts to higher temperatures. Data for individual graphs are obtained from the TG data shown in Figs 2, 3. TG were performed with reduced oxygen access in a nitrogen stream as carrier gas with the rate of flow 50 mL min^{-1}.

The mass loss on the main section was shifted by 10 °C when changing the heating rate by 5 °C min^{-1} and by 20 °C at a speed increase of 10 °C min^{-1}. The main peak of the DTG curve was at the temperature of 340 °C at 5 °C min^{-1}, at the temperature of 360 °C and 390 °C at the heating rate of 10 °C min^{-1} and 20 °C min^{-1}, respectively (Fig. 1). Temperatures of peak DTG correspond to 30–80% conversion, according to heating rate. For sawdust they are at conversion from 50% to 70%, at temperature of 350 °C to 400 °C. The changes in the mass of samples are shown in Fig. 2. Vaporization was carried out till the temperature of 170 °C at the heating rate 5 °C min^{-1}.

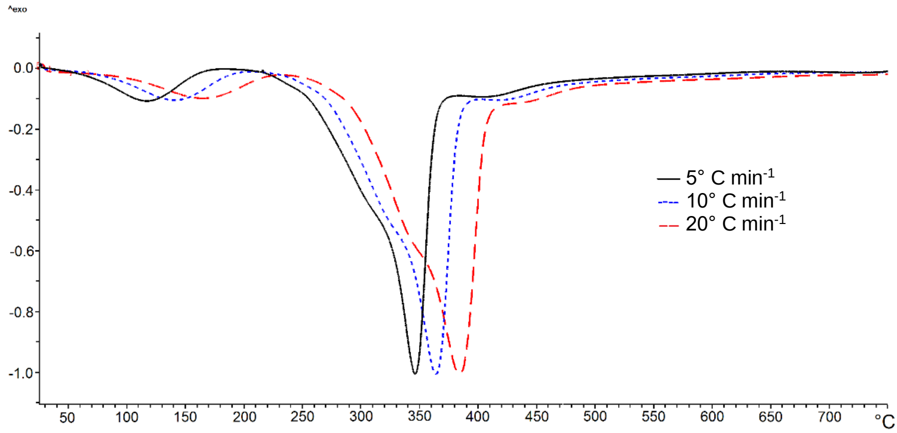


Figure 1. DTG curves for the pellets of degradation 5 °C min^{-1} (solid line), 10 °C min^{-1} (spotted), 20 °C min^{-1} (dashed).

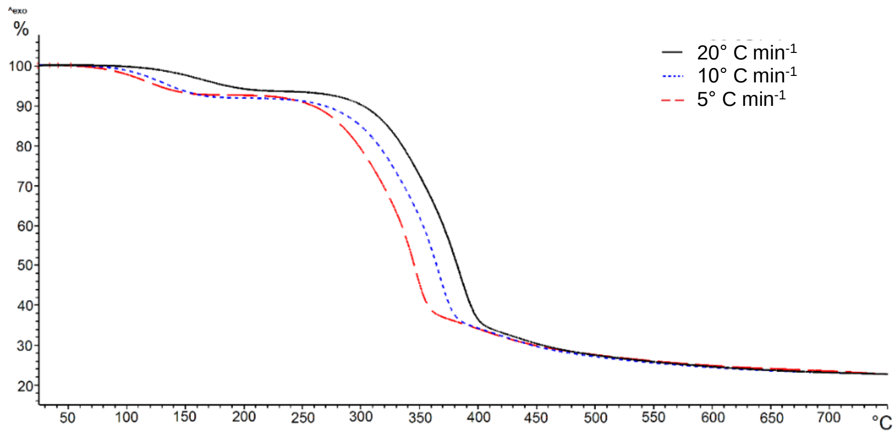


Figure 2. Dependency of mass loss (%) for pellets to temperature, TGA curves of degradation 5 °C min^{-1} (long-dashed lines), 10 °C min^{-1} (short-dashed lines), 20 °C min^{-1} (solid line).

The evaporation process is finished at approximately 210 °C at the heating rate 20 °C min^{-1}. The main decomposition of the hemicellulose material occurs at temperature of 250 °C to 400 °C, a decrease from above 500 °C is attributed to the decomposition process of the secondary pyrolysis products.

The DTG curve shows two main mass changes at heating rate 20 °C min^{-1} at temperatures around 150 °C and 370 °C. For the heating rate of 5 °C min^{-1}, these temperatures are 120 °C and 350 °C where evaporation and major biomass decomposition occur. The decomposition peak of approximately 350 °C is little shifted to the right side, which corresponds to another decomposition process change in mass. This decrease covers a temperature range from 250 °C to 350 °C, with a peak at 300 °C. For higher heating speeds, these speeds move to higher temperatures (Damartizs et al., 2011; Ali et al., 2017). This overlapping process corresponds to the breakdown of cellulose.

The major decomposition peak in the temperature range from 300 °C to 450 °C has a peak at 345 °C and corresponds to the breakdown of hemicellulose bonds. As expected, when the heating rate is increased, these peaks are shifted to higher temperatures. Decomposition peaks correspond to temperatures of 365 °C and 385 °C for the heating rate of 10 °C min^{-1} and 20 °C min^{-1}, respectively. For sawdust, the temperature range is approximately same for the pellets at the heating rate of 10 °C min^{-1} and 20 °C min^{-1}. Small difference was found in heating rate of 5 °C min^{-1}, this decomposition is shifted to lower temperatures with a peak at 325 °C (Fig. 3).

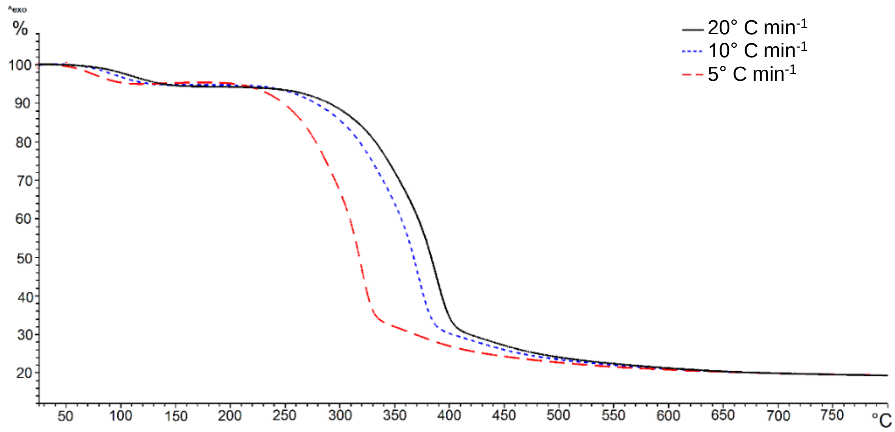


Figure 3. Dependency of mass loss (%) for sawdust to temperature, TGA curves of degradation at 5 °C min^{-1} (solid line), 10 °C min^{-1} (dashed), 20 °C min^{-1} (spotted).

Conversion rate temperature was determined from TG curves. Individual activation energies for a conversion range from 0.1 to 0.9 were determined from the slope of the trend line and Eqs 3, 4 were used to create Figs 4–7.

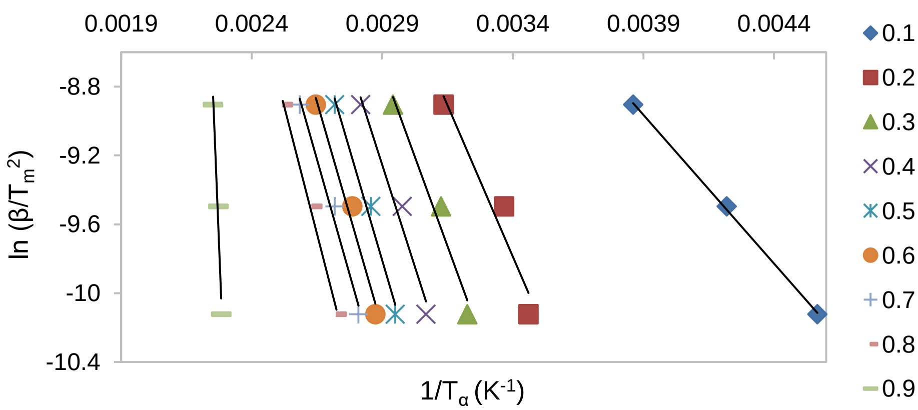


Figure 4. Typical linear regression lines of iso-conversional methods at individual conversions for pellets by KAS method.

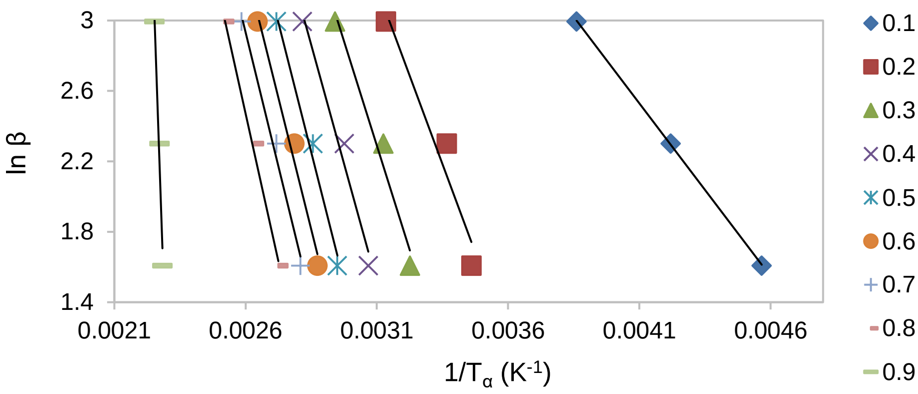


Figure 5. Typical linear regression lines of iso-conversional methods at individual conversions for pellets by FWO method.

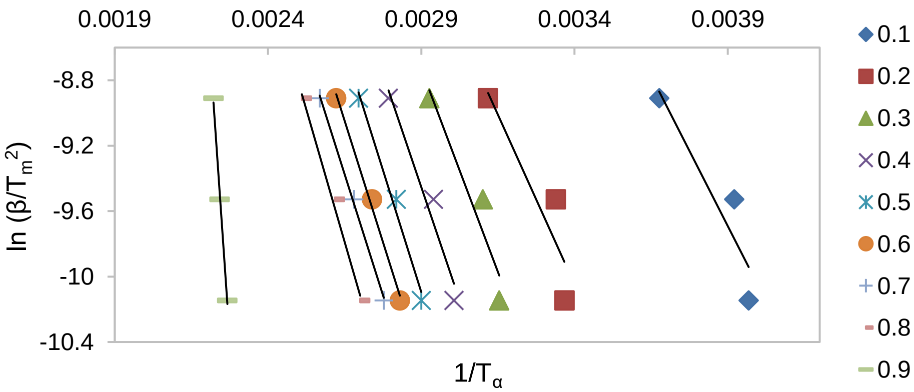


Figure 6. Typical linear regression lines of iso-conversional methods at individual conversions for sawdust by KAS method.

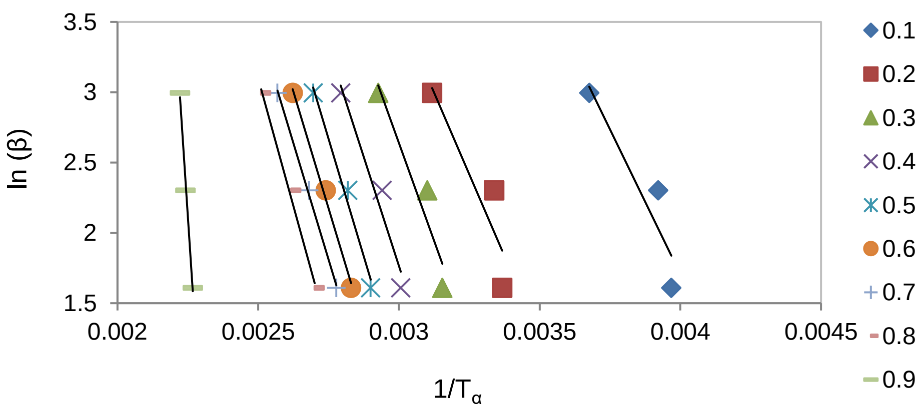


Figure 7. Typical linear regression lines of iso-conversional methods at individual conversions for sawdust by FWO method.

Conversion rate 0.9 was not used in comparison because big increase of activation energy occurred. The activation energy of the pellet for the individual conversion values ranges from 14.36 kJ mol^{-1} to 48.91 kJ mol^{-1} for the KAS model. For the FWO model, these values are not very different and range from 15.54 kJ mol^{-1} to 53.05 kJ mol^{-1}, with a conversion from 10% to 80%. Specific values for each model and conversion value are shown in Table 1. For sawdust, the activation energy ranges from 30.56 kJ mol^{-1} to 53.82 kJ mol^{-1} for the KAS model. The lowest activation energy is for 10% conversion and the highest value at 80% conversion. For the FWO model, the activation energy was from 32.54 kJ mol^{-1} to 57.30 kJ mol^{-1}, for the conversion of 10% to 80%. All values of activation energy for individual models and samples are shown in Table 1, where α is taken as conversion from 10% to 90%.

Table 1. Overview of the obtained activation energies

α	Pellets E_a KAS (kJ mol^{-1})	Pellets E_a FWO (kJ mol^{-1})	Sawdust E_a KAS (kJ mol^{-1})	Sawdust E_a FWO (kJ mol^{-1})
0.1	14.36	15.54	30.56	32.54
0.2	29.21	31.73	34.47	36.70
0.3	34.50	37.43	41.29	43.96
0.4	39.34	42.67	46.02	48.99
0.5	42.89	46.49	49.57	52.77
0.6	43.55	47.22	49.30	52.48
0.7	44.41	48.13	49.14	52.31
0.8	48.91	53.05	53.82	57.29
0.9	315.93	342.89	225.84	217.91

Activation energy increases with the degree of conversion increase (Table 1). The final value for conversion of pellets resp. sawdust for 90% is higher about 267.02 kJ mol^{-1} resp. 172.02 kJ mol^{-1} for the KAS model and about 289.84 kJ mol^{-1} resp. 160.62 kJ mol^{-1} for FWO model. The energy value for conversion 0.9 is different for both models and both are not included in the overall activation energy evaluation due to the inaccurate character of the process description.

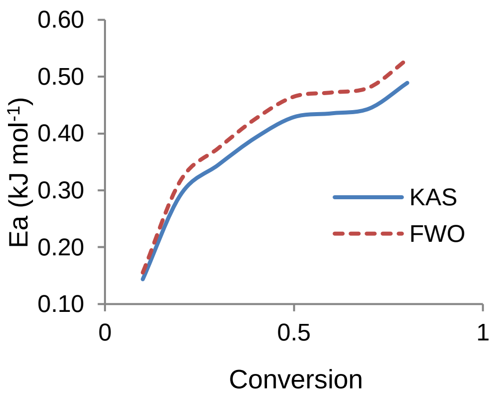


Figure 8. Activation energies of pyrolytic process for pellets.

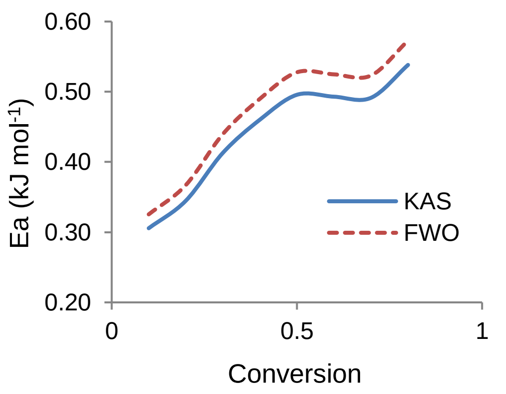


Figure 9. Activation energies of pyrolytic process for sawdust.

Conversion dependencies of activation energy for both wood samples are shown in Figs 8 and 9. It is possible to observe from Fig. 8 and Fig. 9 that activation energy of pellets and sawdust increases with increasing conversion. Activation energy increases with conversion degree for both sawdust and pellets (Table 1). In the conversion zone from 0.55 to 0.8 a less large increase is observed. It can be ascribed to the fact that there existed composed samples (Bartocci et al., 2019). The activation energy of the pellets is in every case between 1.5% and 18.1% for the KAS model and 1.6% to 17% for the FWO model, for the same conversions of material. The highest difference between the models 4.67% was obtained in the case of pellets for the conversion of 0.2.

Authors Wu et al. (2019) explored activation energy of pellets at the gasification. Their values of activation energy ranged from 8.77 kJ mol^{-1} to 59.57 kJ mol^{-1} for individual gaseous products. Activation energy values of pellets and wood powder varied from 92.33 kJ mol^{-1} to 71.20 kJ mol^{-1} (Haobin et al., 2018). Authors Guo et al. (2014) determined the activation energy for fresh wood pellets in ranges from 50 kJ mol^{-1} to 60 kJ mol^{-1}. We obtained similar activation energy by TGA experiments for sawdust and pellets. It can be seen from Figs 8 and 9 that the activation energy of the KAS model is lower than for the FWO. The progress of activation energy is the same for both methods. The same character of the activation energy with increasing conversion is reported in literature from authors Ali et al (2017). The energy value for conversion 0.9 is different for both models and both are not included in the overall activation energy evaluation due to the inaccurate character of the process description.

CONCLUSION

The behaviour of ligninocellulosic material in the form of sawdust and pellets was studied during thermal degradation without access of air by using TGA. The design of the TGA curve corresponds to the shape of the curve for biomass with different contents of cellulose, hemicellulose and lignin. Cellulose decomposed in a narrow range of temperatures, covered by the decomposition of other components corresponding to the wood biomass composition. The biggest mass loss corresponding to the thermal degradation is evident at temperatures from 250 °C to 400 °C, depending on the heating rate. At these temperatures, the decomposition of cellulose, hemicellulose and lignin occurs in two parts. Temperature shifts to higher temperatures at higher heating rates are expected and may be due to heat transfer in the sample. The determination of the activation energy was based on iso-conversional methods that are most suitable to determine the activation energy for samples of biomass. We also found out that values of activation energy of pellets and sawdust are different. This can be caused by different composition of materials. For heat treatment of wood materials, it is necessary to know their kinetics parameters and the results can be used for further understanding of pyrolysis process. Due to the structural changes in sawdust during pelleting, we assume change of the activation energy. Links created due to the compaction could result in a decrease of the activation energy.

ACKNOWLEDGEMENTS. This work was supported by project SUA Grant Agency: Thermal analysis of selected components of sorted waste, no. 10-GA-SPU-17 and project KEGA the project Multimedia Textbook of Physics for Engineers, no. 017SPU-4/2017.

REFERENCES

Ali, I., Bahaitham, H. & Naebulharam, R. 2017. A comprehensive kinetics study of coconut shellwaste pyrolysis. *Bioresource Technol.* **235**, 1–11.

Bach, Q., Chen, W., Eng, Ch., Wang, Ch., Liang, K. & Kuo, J. 2019. Pyrolysis characteristics and non-isothermal torrefaction kinetics of industrial solid wastes. *Fuel.* **251**, 118–125.

Blaschke, T., Biberacher, M., Gadocha, S. & Schardinger, I. 2013. 'Energy landscapes': Meetingenergy demands and human aspirations. *Biomass Bioenerg*. **55**, 3–16.

Bartocci, P., Tschentscher, R., Stensrod, R. E., Barbanera, M. & Fantozzi, F. 2019. Kinetic Analysis of Digestate Slow Pyrolysis with the Application of the Master-Plots Method and Independent Parallel Reactions Scheme. *Molecules* **24**(9), 1657–1672.

Damartizs, Th., Vamvuka, D., Sfakiotakis, S. & Zabaniotou, A. 2011. Thermal degradation studies and kinetic modeling of cardoon (Cynara cardunculus) pyrolysis using thermogravimetric analysis (TGA). *Bioresource Technolog*. **102**(10), 6230–6238.

Fernandez, A., Mazza, G. & ROdrigueza, R. 2018. Thermal decomposition under oxidativeatmosphere of lignocellulosic wastes: Different kinetic methods application. *J Environ Eng*. **6**, 404–415.

Flynn, J.H. & Wall, L.A. 1966. A quick, direct method for the determination of activation energyfrom thermogravimetric data. *J Polym Sci Pol Lett*. **4**, 323–328.

Guo, W., Trischuk, K., Bi, X., Lim, C.J. & Sokhansan, S. 2014. Measurements of wood pelletsself-heating kinetic parameters using isothermal calorimetry. *Biomass Bioenerg*. **63**, 1–9.

Haobin, P., Li, Y., Li, Y., Yuan, F. & Chen, G. 2018. Experimental Investigation of CombustionKinetics of Wood Powder and Pellet. *J. Combust*. **2018**, 7 pp.

Hlaváč, P., Božiková, M. & Cviklovič, V. 2016. Dynamic viscosity and activation energy of wortduring fermentation and storing. *Acta Technologica Agriculturae* **19**(1), 6–9.

Hosoya, T., Kawamoto, H. & Saka, S. 2007. Cellulose–hemicellulose and cellulose–lignininteractions in wood pyrolysis at gasification temperature. *J. Anal. Appl. Pyrol.* **80**, 118–125.

Hu, M., Wang, X., ChEn, J., Yang, P., LIu, C., Xia, B. & Gu, D. 2017. Kinetic study and syngasproduction from pyrolysis of forestry waste. *Energ. Convers. Manage.* **135**, 453–462.

Jouhara, H., Ahmad, D., Boogaert, I., Katsou, E., Simons, S. & Spencer, N. 2018. Pyrolysis ofdomestic based feedstock at temperatures up to 300 °C. *Thermal Science and Engineering Progress* **5**, 117–143.

Chan, Y.H., Yusup, S., Quitain, A.T., Tan, R.R., Sasaki, M., Lam, H.L. & Uemura, Y. 2015. Effect of process parameters on hydrothermal liquefaction of oil palm biomass for bio-oil production and its life cycle assessment. *Energ. Convers. Manage.* **104**, 180–188.

Liu, Z. & Han, G. 2015. Production of solid fuel biochar from waste biomass by low temperaturepyrolysis. *Fuel* **158**, 159–165.

Nilova, L., Naumenko, N. & Kalinina, I. 2017. A study of the forms of bound water in bread andbakery products using differential thermal analysis. *Agronomy Research* **15**(S2), 1368–1398.

Ondro, T., Húlan, T. & Vitázek, I. 2017. Non-isothermal Kinetic An alysis of the Dehydroxylation of Kaolinite in Dynamic Air Atmosphere. *Acta Technologica Agriculturae* **20**(2), 52–56.

Rana, R., Nanda, S., Meda, V., Dalai, A.K. & Kozinski, J.A. 2018. A Review of Lignin Chemistry and its Biorefining Conversion Technologies. *Journal of Biochemical Engineering and Bioprocess Technology* **1**(2), 1–2.

Starkin, M.J. 2003. The determination of activation energy from linear heating rate experiments: a comparison of the accuracy of isoconversion methods. *Thermochim Acta* **404**(1–2), 163–176.

Soria-Verdugo, A., Garcia-Hernando, N., Garcia-Gutierrez, L.M. & Ruiz-Rivas, U. 2013. Analysis of biomass and sewage sludge devolatilization using the distributed activation energy model. *Energ. Convers. Manage*. **65**, 239–244.

Suriapparao, D.V., Ojha, D.K., Ray, T. & Vinu, R. 2014. Kinetic analysis of co-pyrolysis ofcellulose and polypropylene. *J Therm Anal Calorim*. **117**, 1441–1451.

Suzdalenko, V., Gedrovics, M. & Vitolins, V. 2014. Experimental pilot device for thermal analysis of biomass co-firing. *Agronomy Research* **12**(2), 611–622.

Tamelová, B., Malaťák, J. & Velebi, J. 2019. Hydrothermal carbonization and toreffaction ofcabbate waste. *Agronomy Research* **17**(3), 862–871.

Tomei, J. & Helliwell, R. 2016. Food versus fuel? Going beyond biofuels. *Land Use Polic*. **56**, 320–326.

Vassilev, S.V., Baxter, D., Andersen, L.K. & Vassileva, Ch.G. 2010. An overview of the chemical composition of biomass. *Fuel* **89**, 913–933.

Vyazovkin, S., Burnham, A.K., Criado, J.M., Pérez–Maqueda, L.A., Popescu, C. & Sbirazzuoli, N. 2011. ICTAC kinetics committee recommendations for performing kinetic computations on thermal analysis data. *Thermochim Acta*. **520**, 1–19.

Vyazovkin, S. & Sbirrazzuoli, N. 2006. Isoconversional Kinetic Analysis of Thermally Stimulated Processes in Polymers. *Macromol Rapid Comn*. **27**, 1515–1532.

Wu, Z., Zhang, B., Hu, H., Hao, W., Ma, Ch., Li, Y. & Yang, B. 2019. Products distribution andkinetic analysis on gaseous products during fast pyrolysis of two kinds of biomass pellet. *Fuel* **249**, 8–14.

Zhengang, L. & Guanghua, H. 2015. Production of solid fuel biochar from waste biomass by low temperature pyrolysis. *Fuel* **158**, 159–165.

Agronomy Research **17**(6), 2317–2326, 2019
https://doi.org/10.15159/AR.19.205

Technology development of obtaining essential fatty acids from hydrobionts hydrolyzates

E. Kuprina*, V. Filipov*, A. Malova, V. Abramzon, A. Lepeshkin and M. Chikisheva

ITMO University, Faculty of Food Biotechnologies and Engineering, Lomonosov street 9, RU191002 Saint-Petersburg, Russia
*Correspondence: elkuprina@yandex.ru; valery98rus@mail.ru

Abstract. ω-3, 6-fatty acids from hydrobiontsare a minor component in the nutrition of European countries population. This causes a number of diseases, such as cardiovascular ones, cancer etc. There is a task of concentrating these acids in oil due to the fact that to meet their daily needs it is problematic to use large quantities of fish oil-from 15 to 20 g. Particularly rich in ω-3, 6-acids are wastes from the cutting of hydrobionts, containing muscle tissue and skin.
Protein hydrolysates were obtained from rainbow trout (*Oncorhynchusmykiss*) and Atlantic herring (*Clupeaharengus*) wastes by the electrochemical method using electrolyzers of the original design which are allowed to be used in food industry. A technological scheme of separating of lipids from protein hydrolyzates has been developed and experimental batches of oil samples have been developed. To concentrate the fatty acids the cryoconcentration method was used. The phase transitions of the obtained lipids were studied after their cryoconcentration in the temperature range from + 15 °C to minus 40 °C in the environment of calcium chloride using a low-temperature refrigeration unit. To analyze phase transitions the plant was used, which is a container with a solution of calcium chloride cooled by a low-temperature refrigeration machine. The properties of 5 fractions of lipids formed at the time of lipid phase transitions have been identified and studied (the fractional composition, acid, iodine numbers, the content of polyunsaturated fatty acids (PUFAs), vitamin D_3 and A).
It was established that as cryoconcentration increases the concentration of PUFAs, reaching values close to 90%, which allows the resulting product to be attributed to biologically active food additives (BAA). By calculation, it was shown that to create functional food products on fish base from fish of the Gadidae family it is enough to inject 4 grams of BAA to 100 grams of the product. Organoleptic properties of food products from low-fat fish species were improved.

Key words: fish oil, omega-3-fatty acids, omega-6-fatty acids, functional food, cryoconcentration, biologically active food additives.

INTRODUCTION

Currently, there is an acute shortage of ω-3 and ω-6-unsaturated fatty acids in the daily ration of most European countries, since their main dietary sources – fatty sea fish, seafood (Lee et al., 2009, Ryckebosch et al., 2012) – are not common food for people in mainland countries.

Structural components of lipids prevent the deposition of low-density lipoproteins and cholesterol in the blood vessels walls, prevent the aggregation of blood cells and the formation of blood clots, relieve inflammations, etc. (Caterina et al., 2006). In the absence of these essential nutritional factors, severe cardiovascular diseases, heart attacks, strokes occur, and the life expectancy of the population is reduced.ω-3alsohave been used in the management of diabetes and arthritis (Weylandt & Kang, 2005; Hurst et al., 2010; Adeyemi & Olayaki, 2018). The consumption of polyunsaturated fatty acids (PUFA) as an advice of dietary guideline recommendations replaces saturated fatty acids for the prevention of cardiovascular disease (Chowdhury & Steur, 2015). Importantly, the richest sources of omega-3 PUFA (ω-3 FA) are marine oils, which consumption have been linked to cardiovascular protection (Nogueira, et.al., 2018). Besides, it was shown that ω-3 FA from fish oil is more potent then plant ω-3 FA to inhibit mammary tumors, the problem which is world-spread nowadays (Liu et al., 2018).

Numerous studies show that in addition to hydrobionts themselves, a large amount of essential fatty acids is also contained in the wastes from their cutting. Therefore, it is advisable to use them to obtain BAA in the form of concentrated solutions of unsaturated fatty acids. To develop the technology, the biochemical composition of the salmon and herring was studied and the feasibility of their use was shown.

It is known that to obtain lipid-derived BAA from oily fish raw materials saturated with omega-3 and omega-6 acids, vitamins and phospholipids, technologies based on the hydrolysis of protein components of the raw material, followed by the release of oil components from the protein-lipid emulsion, have the advantage; for example, technology of producing a vitamin A concentrate (Honold et al., 2016; Ghelichi et al., 2017; Najm et al., 2017).

Among the existing technologies of the hydrolysis of the waste from the processing of hydrobionts, namely, acid-alkaline, enzymatic, etc., was selected alkaline hydrolysis technology, based on the synthesis of alkali in the process of electrochemical treatment of raw materials (Gajanan, 2016; Hleap-Zapata & Gutiérrez-Castañeda, 2017). The electrochemical processing parameters were developed by us earlier for the isolation of protein from crustaceans (Kuprina et al., 2015). The technology makes it possible to combine usage of a non-reactionary solution with the direct effect of a constant electric field on raw materials, which ensures the rapid achievement of the process of dissolution and hydrolysis of dispersed raw materials at low concentrations of hydroxyl ions and preserving the quality of nutrients. Lipids are separated from the protein solution by separation using standard equipment.

The fatty acid composition of the isolated lipids was investigated and the presence of a large number of ω-3 fatty acids in them was shown. The special value of lipids isolated from protein hydrolysates is due to the fact that lipids of muscle tissues (in particular, waste from cutting of hydrobionts), unlike lipids contained in the internal organs of hydrobionts, contain substances that are highly biologically valuable (phospholipids, ω-3,6-fatty acids, vitamins), while in the internal organs triglycerides prevail. Despite the difficulties in the separation of ω-3,6-fatty acids (due to the connection with proteins in the form of lipoprotein complexes), we conducted studies on their preparation from lipids as the target product in the framework of the electrochemical technology for processing raw materials (Jacobsen, 2012; García-Morenoet al., 2013).

Concentration of the resulting oil components is promising due to the problematic intake of large amounts (15–20 g per day) of traditional dietary supplements-fish oil, due to its fatty and specific consistency (Drusch, 2012). In this regard, particular interest is in the development of technology for the extraction of essential fatty acids from fish oil, in particular by the method of cryoconcentration, which allows preserving the quality of fat thanks to the use of low-temperature regimes, which significantly slow down the oxidation processes.

MATERIALS AND METHODS

Sampling

The following raw materials were chosen as objects of research for the production of protein-fat emulsion (hydrolyzate): rainbow trout (*Oncorhynchus mykiss*) and Atlantic herring (*Clupea harengus*). As a sample for cryoconcentration was taken the fat obtained by separation from the emulsion.

Raw fish was obtained in chilled (trout) and frozen (herring) form. The trout was transported in hermetically packed plastic containers. Storage was carried out in a refrigerating chamber at a temperature of + 5 °C. Frozen herring was delivered in blocks, storage was carried out in a freezer at a temperature of minus 18 °C.

Electrochemical method of obtaining protein hydrolyzate

The process of electrochemical processing includes certain stages: swelling, extraction of water-soluble components (albumin, carbohydrates, etc.) and extraction of difficult-to-dissolve components (myofibrillary and other proteins, protein-lipid, protein-glycoside, and other complexes). The process completes by the transition of proteins, polypeptides, lipids into a solution in the form of an emulsion and precipitation of bone or crustaceous tissues. The selected method has several advantages:

- high yield (95–98%) of lipids from raw materials;
- carrying out simultaneously the extraction of fat and its refining (due to processing in the cathode chamber of the electrolyzer at pH values ≥ 12.2);
- preservation of high quality of the obtained lipids due to gentle processing modes (since they are not exposed to long-lasting high temperatures, pressure (as in the press-drying technology) or solvents.

Due to the fact that the fat, isolated from protein solutions, is characterized by high values of pH, a stage of its neutralization is necessary. The first stage of hydration is washing with 10% sodium chloride solution and hot water (temperature 90–100 °C) at a ratio of 1:1 until complete removal of alkali and soap. The number of washes ranges from 3 to 5. Each washing is completed by mixing the mass for 10–15 minutes, settling for 1–2 hours and draining the bottom of the sludge. The top is washed again.

To remove remaining moisture the washed fat is dried at a temperature of about 140 °C and in vacuum of at least 79.98 kPa during non-stop work of the stirrer. After drying, the fat should not contain more than 0.6% moisture. Then the fat is sent to the separation.

Physico-chemical analysis

Physico-chemical properties of lipids were determined according to GOST 7636-85, namely, iodide and acid numbers.

Determination of the Fatty acid composition of lipids

The fatty acid composition was investigated by gas chromatography method (with preliminary methylation of the samples). The obtained samples of fish oil were investigated by chromatographic method (Godoy & Rodriguez-Amaya, 1993). Analysis of the qualitative composition of fish oil was carried out on gas chromatographic mass spectrometer (Shimadzu GCMS-TQ8040). The collection and processing of data was carried out using the software of the indicated device. When establishing the calibration characteristics and performing measurements of the mass fraction of fatty acids, certain conditions were observed.

Fractional division of lipids by cryoconcentration

Fish oil was cooled in glass tubes with a volume of 50 mL, placed in a container with a 28% solution of calcium chloride, cooled by low-temperature refrigeration unit. The temperature was measured inside the sample and in a cooling medium with electronic thermometers of the 'Vapan' brand. After fixing the phase transitions in the oil at temperatures of +4, minus 6, minus 14 and minus 37 °C, the fat was mixed with acetone in a ratio of 1:8 and then repeated cooling from +20 to minus 40 °C. After each phase transition the oil was separated into solid and liquid fractions by filtration.

The reliability of the data was achieved by planning the number of experiments necessary and sufficient to achieve a confidence level in scientific experiments $P = 0.95$. Statistical data processing was performed using standard methods for evaluating test results for small samples using Microsoft Excel 2010.

RESULTS AND DISCUSSION

It is known that fish raw materials contain a number of valuable biologically active substances of lipoid nature, namely: essential polyunsaturated omega-3 and omega-6 fatty acids, vitamins A, D and E, phospholipids.

Currently, there is a tendency to obtain biologically active food supplements based on fish oils from the fatty fish processing waste. The waste composition from fish cutting is presented in Table 1.

Table 1. Chemical waste composition from cutting of Atlantic herring *Clupea harengus* and rainbow trout *Oncorhynchus mykiss*. Differences between herring and trout samples are statistically significant

Component	Moisture, %	Fat, %	Protein, %	Ash, %	Energyvalue, kcal
	Atlantic herring				
Meat with skin	69.0 ± 0.70	7.3 ± 0.39	22.6 ± 0.37	1.8 ± 0.03	161.0
Bones	58.6 ± 0.89	10.6 ± 0.33	18.8 ± 0.27	9.2 ± 0.38	175.7
Fins	65.4 ± 0.95	9.4 ± 0.37	16.7 ± 0.31	10.0 ± 0.21	156.0
Head	69.5 ± 0.98	9.7 ± 0.08	13.2 ± 0.20	4.7 ± 0.40	139.0
	Rainbow trout				
Meat with skin	71.3 ± 0.92	5.3 ± 0.26	22.0 ± 0.88	1.5 ± 0.10	139.0
Bones	62.4 ± 1.13	11.04 ± 0.72	18.2 ± 0.70	9.0 ± 0.40	176.0
Fins	66.4 ± 0.53	6.6 ± 0.44	16.9 ± 0.70	9.75 ± 0.76	130.7
Head	70.8 ± 0.64	10.2 ± 0.70	14.8 ± 0.40	3.4 ± 0.28	156.0

To achieve a confidence probability of measurements of $P = 0.95$ with the number of experiments repeated $n = 3$, the mean square deviation of the arithmetic mean was S = 0.01, the average deviation of the measurements was Sm = 0.0058, with a Student coefficient tst = 4.3, the confidence interval of the arithmetic mean $\Delta x = 0.02$.

From the data of Table 1 it can be seen that waste from fish cutting, like fish themselves, are characterized by valuable chemical composition, high fat content, which indicates the feasibility of using them as a raw material source for obtaining lipid-type biologically active substances. There was no significant difference in the biochemical composition of the skin, bones, fins and heads of the Atlantic herring and the Rainbow trout. Therefore, to extract fat and other dietary supplements, it is possible to use all the waste products listed in Table 1.

To obtain biologically active substances saturated with omega-3 and omega-6 acids, vitamins and phospholipids, from fatty fish raw materials, a technology was chosen based on the electrochemical hydrolysis of protein components of the raw material with subsequent release of fat components from the protein-lipid emulsion (Kirillov & Kuprina, 2014).

The electrochemical method of influencing the biological raw materials includes a direct effect of the electric field on raw materials and the aquatic environment, which allows for fine control of processes, simplifies their automation, and also reduces energy costs. When processing raw materials in an electric field, the processes of diffusion and extraction are accelerated, and the intensity and extent of chemical and physical processes increase. Thanks to all this, there is an intensification of the raw materialsprocessing.

For electrochemical processing, electrolyzers with a plane-parallel arrangement of electrodes separated by an ion-selective membrane were used. The optimal processing parameters of the dispersed raw material were selected, which ensured the complete dissolution of the protein fraction: current, voltage, processing time of the suspension in the electrolyzer, time for heating the suspension after the electrolyzer in a stirred reactor. The insoluble bone residue was separated by centrifugation. Lipids from the protein solution were isolated by separation. The technological scheme of complex waste processing (pegs, fins, bones, scales, skin) from salmon and herring cutting by electrochemical method with obtaining lipids from protein solutions is shown in Fig. 1.

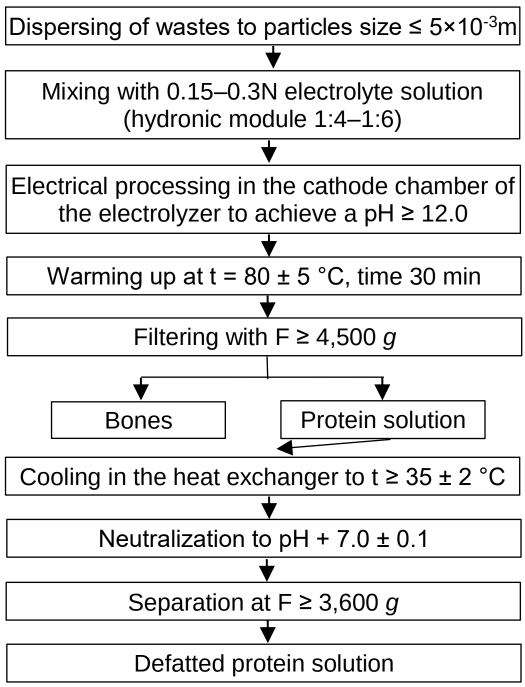


Figure 1. Technological scheme of complex waste processing from fish cutting by electrochemical method with obtaining lipids from protein solutions.

The yield of oil from trout and herring waste cutting during their processing by electrochemical method was 7 and 8%, respectively, which is close to 90% of the theoretical.

This technology was developed with the aim of preserving the quality of biologically active substances of lipoid nature, due to the gentle conditions of exposure to raw materials in the process of extraction of the first. As the existing technologies based on the use of organic solvents, high temperatures, chemical reagents, are distinguished by the ‘hardness’ of the impact on the raw materials, which in turn leads to oxidative deterioration of biologically active substances from lipids and proteins.

The fatty acid composition of the obtained fat was investigated by gas-liquid chromatography and is presented in Table 2.

Table 2. Fatty acid composition of fat samples of herring and trout, % of the amount of fatty acids. Differences between samples are statistically significant

Acid name	Fatty acid index	Average sample herring	Herring protein hydrolyzate fat	Average sample trout
Caprylic	10:0	0.12	0.02	0.02
Lauric	12:0	0.28	0.58	0.25
Myristic	14:0	2.18	5.98	1.15
Myristoleic	14:1	0.06	0.06	0.07
Isopentadecanoic	15:0	0.05	0.16	0.20
Anteiso-pentadecanoic	15:0	0.05	0.05	0.09
Pentadecanoic	15:0	0.26	0.31	0.42
Pentadecenic	15:1	0.05	0.08	0.04
Palmitic	16:0	13.48	10.65	13.15
Hexadecenoic	16:1	0.14	0.12	0.23
Palmitoleic9-cis	16:1	2.55	4.31	3.43
Heptadecylic	17:0	0.22	0.40	0.62
Heptadecenoic	17:1	0.40	0.29	0.49
Stearic	18:0	4.1	3.81	4.84
Elaidic 9-trans	18:1	0.88	0.81	1.13
Oleic 9-cis	18:1	16.80	18.33	20.12
Vaccenic 11-trans	18:1	2.01	1.59	2.05
Octadecenoic 11-cis	18:1	0.09	0.22	0.14
Linoleic ω-6	18:2	3.59	1.08	1.97
γ-linolenic ω-6	18:3	0.17	0.12	0.06
α-linolenic ω-3	18:3	5.80	0.86	0.67
Arachidic	20:0	0.11	0.16	0.21
Gadoleic (∑ isomers)	20:1	6.90	14.31	10.83
Eicosadienoic	20:2	0.33	0.39	0.51
Eicosatrienoic 8, 11, 14-trans	20:3	0.21	0.15	0.23
Arachidonic ω-6	20:4	0.87	0.41	1.08
Eicosapentaenoic ω-3	20:5	6.25	2.3	5.45
Behenic	22:0	0.07	0.06	0.07
Erucic (∑ isomers)	22:1	5.99	15.99	11.23
Docosadienoic	22:2	0.05	0.09	0.13
Docosapentaenoic ω-6	22:5	3.66	3.71	3.54
Docosahexaenoic ω-3	22:6	22.30	7.81	12.03
Lignoceric	24:0	0.04	0.10	0.04
Nervonic	24:1	0.78	0.77	1.24

From the data of Table 2 it follows that the fish oil obtained by the electrochemical method from trout and herring wastes contains a significant amount of omega-3 polyunsaturated fatty acids (about 30% of the total fatty acids), but in quantities insufficient to meet the daily human need (according to MR 2.3.1.2432–08), therefore it was necessary to develop the technology of their concentrating, since the task was to obtain fish oil with a high content of omega-3 acids for subsequent encapsulation.

The fat from the secondary fish raw material was filtered until a transparent, viscous mass without inclusions was obtained and stored at +4 °C. To concentrate fat containing omega-3 acids, the subject of study was cooled. The fat was placed in glass tubes. The process of cooling the fat in test tubes was carried out on the installation, which is a container with a solution of calcium chloride cooled by a low-temperature refrigeration installation. The average rate of cooling and freezing is 0.3 °C s^{-1}. The temperature was fixed inside the sample and in the cooling medium by thermocouples (Drusch, 2012; Ghelichi et al., 2017).

It has been established that phase transitions in fat intensively occur at temperatures: -6 °C, -14 °C, -37 °C. Phase transitions are accompanied by precipitation of lipid fractions that are less saturated with double bonds. After centrifugation, the supernatant lipid fraction enriched in unsaturated fatty acids was further cooled. Fig. 2 shows the photographs of the fractions released during cryoconcentration.

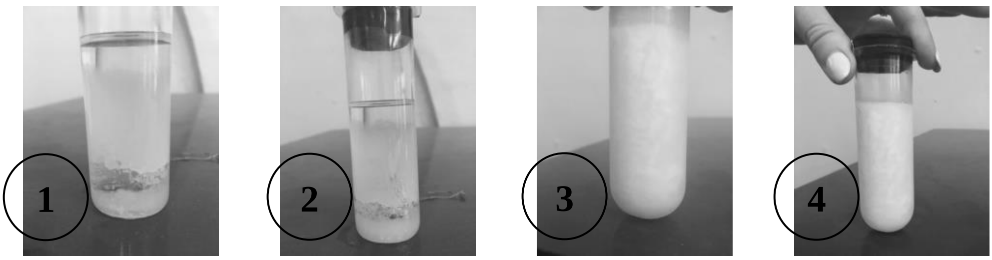


Figure 2. Fat fractions obtained by cryoconcentration of biologically active substances of lipoid nature from secondary fish raw materials. 1 – the photo of the fat sample at 4 °C; 2 – the photo of the liquid fat fraction separated from sample1 at -6 °C; 3 – the photo of the liquid fat fraction separated from sample 2 at -14 °C; 4 – the photo of the liquid fat fraction separated from sample 3 at -37 °C.

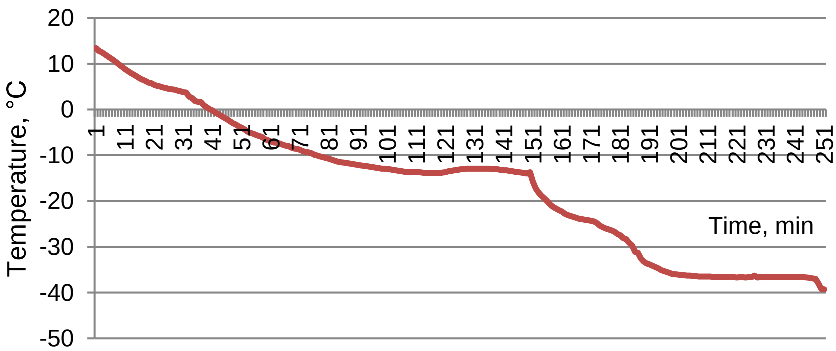


Figure 3. Dependence of temperature in fish oil from the time of its cooling and freezing.

The experiment on cooling and freezing was repeated by mixing fat with acetone in a ratio of 1:8 to allow the quantitative separation of fractions during freezing.

In the course of the experiment of cooling a lipid-containing biologically active substance from a secondary fish raw material, a temperature-time dependence curve was obtained (Fig. 3), where phase transitions and separation of fractions were observed, which corresponds to the data of RF patent No. 2031923.

Temperature measurements were made on a Vapan thermometer with a standard deviation of 0.14. After each phase transition, the precipitated lipoid precipitate was separated and quantified. The results of the fractional analysis are presented in Table 3 and Fig. 4.

Table 3. Dependence of the yield of solid fraction of biologically active substances of lipoid nature from secondary fish raw materials on temperature

Temperature, °C		+4	-14	-37
Separated fraction, %	Experiment 1	6.0	92.5	55.2
	Experiment2	5.1	93.0	56.0

Table 4 presents data on the biochemical characteristics of fat and its sediment obtained at different temperatures.

From the data of Table 4 it follows that due to the process of cryoconcentration, it was possible to increase the content of fat fractions, including omega-3 fatty acids, approximately three times. According to MP 2.3.1.2432-08, the average daily requirement for omega-3 acids is 7.3 g per day. The calculation was carried out taking into account the fact that the daily requirement should correspond to 1–2% of the daily caloric intake (for example, 2,400 kcal for group II of the population, including people under 30 years old with an activity coefficient of 1.6). Considering that Atlantic herring selected for the study contains 1.64 g 100 g^{-1} of omega-3, and trout contains 0.97 g 100 g^{-1}, to meet the daily need for omega-3 acids, herring and trout products need additional enriching with these acids, which can be achieved by introducing into them 22 g of fish oil in the composition of functional food product.

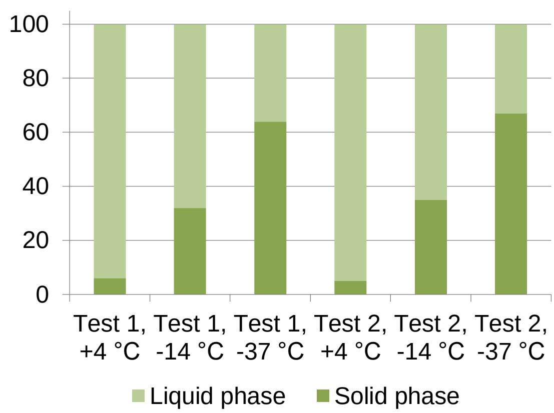


Figure 4. Diagram of the fractional composition of biologically active substances of lipoid nature, obtained from rainbow trout waste cutting.

Table 4. Biochemical properties of cryoconcentrated fat isolated from rainbow trout waste hydrolysate cutting

Indicator	BAS at temperature +4 °C	BAS at temperature -14 °C	BAS at temperature 14 °C	BAS at temperature -37°C
Acid number, mg KOH*g^{-1}	1.6	1.7	2.3	2.5
Iodinenumber, g100 g^{-1}	109.03	298.29	207.52	341.38
Eicosapentaenoic acid, % in fat	0.75	10.00	-	31.3
Docosahexaenoic acid, % in fat	1.80	24.07	-	62.7

However, it is technologically difficult to introduce such a quantity of lipids into functional food products, especially for non-minced products, this drawback can be eliminated by the developed technology of cryoconcentration of lipids. Taking into account the fact that during cryoconcentration the concentration of omega-3 acids increases by 3 times, it is enough to introduce 6 g of fat per 100 g of herring product and 6.6 g of fat per 100 g of trout to meet the daily consumption of omega-3 acids. To meet 30% of the daily consumption of omega-3 acids, the required amount of injected cryoconcentrated fat is up to 1.8 and 1.98 g, respectively, which is technologically easy to implement.

According to FAO and WHO, a food product belongs to the functional food product group, if its consumed portion (100 g) provides a 30% daily consumption rate of the target component.

CONCLUSIONS

Thus, the technology has been developed for obtaining biologically active substances of lipoid nature, enriched with omega-3 acids, from hydrobiont processing waste by the method of electrochemical hydrolysis and cryoconcentration. A comparative analysis of the waste composition from cutting of herring and trout is carried out and the expediency of using them to obtain lipid-type biologically active substances is shown. A process operational diagram has been developed and fat outputs have been determined when it is obtained from fish waste by electrochemical method. The fatty acid composition of the fat obtained by the electrochemical method was determined. It has been established that cryoconcentrated fat obtained from waste from cutting trout and herring using an electrochemical method has a significantly higher content of omega-3 acids and, accordingly, biological value in comparison with edible and medical fish oil from the liver of the Cod family.

It is shown that in order to meet 30% of the recommended daily intake of omega-3 acids in the development of functional food products based on rainbow trout and Atlantic herring, it is necessary to introduce 1.98 g and 1.8 g of cryoconcentrated fish oil. The resulting product is suitable for the enrichment of any fish products with omega-3 acids, and its required amount is determined by calculation, based on the fat content of raw materials. After encapsulation in nanocapsules, the medicine will be suitable for enriching omega-3 acids of any food, which is the subject of further research.

ACKNOWLEDGEMENTS. This work was financially supported by the government of the Russian Federation, Grant RFMEFI58117X0020.

REFERENCES

Adeyemi, W.J. & Olayaki, L.A. 2018. Diclofenac – induced hepatotoxicity: Low dose of omega-3 fatty acids have more protective effects. *Toxicology Reports* **5**, 90–95.

Chowdhury, R. & Steur, M. 2015. Invited commentary: dietary polyunsaturated fatty acids and chronic systemic inflammation – a potentially intriguing link. *American Journal of Epidemiology* **11**, 857–860.

De Caterina, R., Zampolli, A., Del Turco, S., Madonna, R. & Massaro, M. 2006. Nutritional mechanisms that influence cardiovascular disease. *The American Journal of Clinical Nutrition* **2**, 421–426.

Drusch, S. 2012. An industry perspective on the advantages and disadvantages of different fish oil delivery systems. *Encapsulation Technologies and Delivery Systems for Food Ingredients and Nutraceuticals*, pp. 488–504.

Gajanan, P.G., Elavarasan, K. & Shamasundar, B.A. 2016. Bioactive and functional properties of protein hydrolysates from fish frame processing waste using plant proteases. *Environmental Science and Pollution Research* **23**(24), 24901–24911.

García-Moreno, P.J., Pérez-Gálvez, R., Espejo-Carpio, F.J., (...), Guadix, A. & Guadix, E.M. 2013. Lipid characterization and properties of protein hydrolysates obtained from discarded Mediterranean fish species. *Journal of the Science of Food and Agriculture* **93**(15), 3777–3784.

Ghelichi, S., Sørensen, A.-D.M., García-Moreno, P.J., Hajfathalian, M. & Jacobsen, C. 2017. Physical and oxidative stability of fish oil-in-water emulsions fortified with enzymatic hydrolysates from common carp (Cyprinuscarpio) roe. *Food Chemistry* **237**, 1048–1057.

Godoy, H.T. & Rodriguez-Amaya, D. 1993. Evaluation of methodologies for the determination of provitamins A.

GOST 7636–85. *Fish, marine mammals, invertebrates and products of their processing. Methods of analysis*. Interstate Standard for the countries of the Eurasian Economic Union (in Russian).

Hleap-Zapata, J.I. & Gutiérrez-Castañeda, C.A. 2017. Fish hydrolysates-production, profits and new developments in the industry-A review | [Hidrolizados de pescado-producción, beneficios y nuevosavancesen la industria.-Unarevisión]. *Acta Agronomica* **66**(3) 311–322.

Honold, P.J., Nouard, M.-L. & Jacobsen, C. 2016. Fish oil extracted from fish-fillet by-products is weakly linked to the extraction temperatures but strongly linked to the omega-3 content of the raw material. *European Journal of Lipid Science and Technology* **118**(6), 874–884.

Hurst, S., Zainal, Z., Caterson, B., Hughes, C.E. & Harwood, J.L. 2010. Dietary fatty acids and arthritis. *Prostaglandins, Leukotrienes and Essential Fatty Acids (PLEFA)* **4-6**, 315–318.

Jacobsen, C. 2012. Preventing lipid oxidation in omega-3-enriched foods. *International News on Fats, Oils and Related Materials* **23**(3), 138–141.

Kirillov, A.I. & Kuprina, E.E. 2014. Dietary supplement derived from chitin with improved hypolipedemic and adsorption qualities // *XX Conference New Aspects of the Chemistry and Applications of Chitin and its Derivatives*. -Lodz, Poland. 24-26 September 2014.

Kuprina, E.E., Kirillov, A.I., Ishevski, A.L. & Murashev, S.V. 2015. Food supplement based on chitin with enhanced lipid-lowering and sorption properties. *Progress on Chemistry and Application of Chitin and Its Derivatives* **20**, 156–161.

Lee, J.H., O'Keefe, J.H., Lavie, C.J. & Harris, W.S. 2009. Omega-3 fatty acids: Cardiovascular benefits, sources and sustainability. *Nature Reviews Cardiology* **6**(12), 753–758.

Liu, J., Abdelmagid, S.A., Pinelli, C.J., Monk, J.M., Liddle, D.M., Hillyer, L.M., Hucik, B., Silva, A., Subedi, S., Wood, G.A., Robinson, L.E., Muller, W.J. & Ma, D.W.L. 2018. Marine fish oil is more potent than plant-based n-3 polyunsaturated fatty acids in the prevention of mammary tumors. *The Journal of Nutritional Biochemistry* **55**, 41–52.

Najm, S., Löfqvist, C., Hellgren, G., (...), Smith, L.E.H. & Hellström, A. 2017. Effects of a lipid emulsion containing fish oil on polyunsaturated fatty acid profiles, growth and morbidities in extremely premature infants: A randomized controlled trial. *Clinical Nutrition ESPEN* **20**, 17–23.

Nogueira, M.S., Scolaro, B., Milne, G.L. & Castro, I.A. 2018. *LWT* **101**, 113–122.

Patent No. 2031923. *Way to get fish oil.* Closed Joint-Stock Company 'Polien.' of Kramds Center Corporation.

Ryckebosch, E., Bruneel, C., Muylaert, K. & Foubert, I. 2012. Microalgae as an alternative source of omega-3 long chain polyunsaturated fatty acids. *Lipid Technology* **24**(6), 128–130.

Weylandt, K.H. & Kang, J.X. 2005. Rethinking lipid mediators. *The Lancet* **366**, 618–620.

Agronomy Research **17**(6), 2327–2338, 2019
https://doi.org/10.15159/AR.19.196

Planting and tending productivity comparison in mounds and disc trenches using containerized and bareroot coniferous seedlings

K. Makovskis*, D. Lazdina, S. Kaleja, G. Spalva and K. Dumins

Latvian State Forest Research Institute "Silava", 111 Riga street, LV–2169 Salaspils, Latvia
*Correspondence: kristaps.makovskis@silava.lv

Abstract. In 2016 more then 40,300 ha of forest was regenerated in Latvia, where 13,000 ha were seeded or planted and 30,300 ha were left in natural regeneration. Before planting, usually one of two soil preparation methods are used – mounding or disc trenching. In areas with optimal water regime, disc trenching is used, while in wet areas mounding is used. Tree planting and after planting tending is done manually by hand tools. The aim of the study was to compare planting and tending productivity in different soil preparation methods (mounding and disc trenching) by planting different stocktypes (containerized and bareroot seedlings). Planting time studies were done in 12 sites and tending time studies in 8 sites. In planting time studies, different planting operations were measured and compared. In tending time studies, GPS devices were used, where area, distance and working time (productive and rest) was counted from GPS data. Average planting time for containerized seedlings in disc trenches was 10.3 seconds, while in mounds 9.2 seconds per seedling, an 11% improvement. Average planting time for bareroot seedlings in mounds was 28.3 seconds, while in trenches – 18.2 seconds, a 35% improvement. Tending in trenches was done faster than in mounds. On average, one hectare tending time in mounds was 8.4 hours, while in trenches 7.4 hours, an 11% improvement. Walked distance for 1 hectare tending in mounds was 5.4 km, 7% shorter than the distance of 5.0 km in trenches. Factors that influence planting and tending productivity are soil preparation quality, logging residue, and water level on the site. Data from planting and tending time studies could be used for better plan work activities and select suitable planting material for a particular soil preparation method.

Key words: planting time studies, planting productivity, tending time studies, tending productivity, planting in mounds, planting in trenches.

INTRODUCTION

Forest in Latvia cover 3.3 million hectares of land, or 52% of the country territory. In 2016 more than 40,300 ha of forest was regenerated in Latvia, where 13,000 ha were seeded or planted (artificial regeneration) and 30,300 ha were allowed to regenerate naturally (Central Statistical Bureau of Latvia 2018). Natural regeneration occurs by sprouting or leaving the seed trees on the cleared area. Artificial regeneration happens through planting or seeding and consists of soil preparation, planting, and later tending. Planted forest stands, in most cases, have higher productivity compared to naturally regenerated, achieved through use of tree breeding and better soil preparation (Nordborg et al., 2003; Heiskanen et al., 2013; Jansons et al., 2015). Before planting two main soil preparation methods are used: mounding and disc trenching (Fig. 1).

Mounds Disc trenches

Figure 1. Examples of the forest soil preparation methods used in Latvia before planting. These site preparation techniques are done during the autumn before planting (photos from our study sites).

In mounding, a hole with excavator bucket is scoped into the soil, turned upside down and placed next to the hole. The new pile is called a mound. In trenching, a disc is towed behind forest tractor to form a long trench. Soil preparation with trenchers is cheaper, faster, and more widely used compared to mounding method. On average, trencher productivity for one hectare of soil preparation is 1.0–1.8 hours (UOT-2000 Forest Trencher, 2019), while in mounding, when 2,000 planting spots per hectare are prepared, average productivity is 5.9–6.9 hours (Lazdina et al., 2018). Average trenching service costs are 120–180 EUR ha^{-1}, while mounding costs 450–550 EUR ha^{-1} (Lazdina, 2017). Besides using seeds during direct seeding, three different seedling types are used in artificial regeneration: bareroot, bareroot-container hybrids, and containerized seedlings (Latvian State Forests, 2015). Bareroot seedlings have an open root system that is not specially designed. These seedlings have a limited planting time, and the possibility of roots drying in the soil is not ruled out. Bareroot-container hybrid seedlings have an open, vertically oriented, compact root system. During the first half of the year they are grown as containerized seedlings in a greenhouse and in the second half of the year (middle of summer) they are transplanted to an open field where they are grown an additional one or two years. As a result, these seedlings have a robust root system that accelerates plant growth in the first years after planting. The possibility of roots drying out is very low, but these seedlings also have a limited planting time. Containerized seedlings have a closed, vertically oriented, compact root system included within the soilless (peat) substrate. Containerized seedlings can be planted almost through the whole vegetation period and seedlings do not dry out when transported and planted. Joint Stock Company ‘Latvia’s State Forest’ with 9 nurseries is the main seedling producer in country. Of the 49.9 mil. seedlings grown in 2017, 8% were bareroot, 43% were bareroot-container hybrids, and 49% were containerized seedlings.

All planting is done manually with planting tubes for containerized seedlings and by spades for bareroot seedlings. In tending all competing vegetation that suppresses tree growth is removed using bush saws. Average planting service costs in 2017 were 85–120 EUR ha^{-1} and tending 124–160 EUR ha^{-1} depending on location and forest type (Central Statistical Bureau of Latvia, 2018).

Previous time studies of forest establishment in Latvia were more related to planting mechanization (Liepins et al., 2011; Lazdina et al., 2018) and early thinning operations (Lazdins et al., 2013; Lazdins et al., 2016; Petaja et al., 2018). In one such study, where tending in similar conditions was done, the main conclusion was that tending on sites prepared by mounding was as effective as on sites where disc trenching was used, and speed of the operation was unaffected by soil preparation method (Dzerina et al., 2016). This study did not, however, analyze time spent for different operations of manual planting and or the distance of walking during tending operation.

The planting cycle consists of actions or elements that directly make up the act of planting a tree and interruptions that occur as planting proceeds. Some undesirable planting activities are also outside the planting cycle, but remain to overall affect planting productivity (Vyse, 1973). Actions such as site preparation, planting, stumping, and walking in site are elements that are directly connected to tree planting. Pauses and other breaks are not directly related to planting, but will affect planting if they continue for a longer time period. Other elements, like driving to the site, driving between different sites, or seedling transportation to the site can influence productivity over a longer time period.

In Finland, approximately 60% of the conifer forest stands are judged to require early cleaning (substantial 37.2%; high 21.2%) (Uotila et al., 2012). In Latvia, where forest soils are more fertile, tending mostly starts in the year of reforestation if the competitive overgrowth (canopy competition) interferes with successful tree growth. Tending continues several years after planting, one or more times per year, depending on overgrowth intensity. Tending intensity depends on tree species, soil fertility, and weather conditions.

Main tending productivity influencing factors are overgrowth intensity, tree species, forest type, and working methodology (Zimelis et al., 2011). In Finnish forests, early cleaning or tending substantially reduced canopy competition and, consequently, the mean diameter of released spruce grew 21–32% faster depending on the site. Finnish forest scientists reported that tending activity can reduce the cost of pre-commercial thinning, because tending reduces the estimated time needed for subsequent management by 18–49% and offers an economically viable young stand management option (Uotila & Saksa, 2014). To ensure high quality of stands at the felling age, intense thinning of young stands should be used (Zālītis et al., 2017). Despite the fact that mounding is more costly than disc trenching, at the interest rate of 3%, the investment in spot mounding had a 329 EUR ha^{-1} higher net present value than the investment in disc trenching (Uotila et al., 2010).

MATERIALS AND METHODS

On our study sites, disc trenching and mounding soil preparation methods were used. Study sites were located in the central part of Latvia in JSC 'Latvian State Forest' managed areas. In sites with optimal water regime, trenching was was done with a forest machine with attached disc trencher. On wet sites, mounding was down with an excavator and a conventional bucket. In total 12 sites were chosen, where soil preparation was done in the previous autumn.

Planting time studies

Planting was done on 12 sites. On 6 sites the soil preparation method was mounding and on 6 sites it was disc trenching. In each soil preparation method, 3 sites were reforested with pine (*Pinus sylvestris*) and 3 sites with spruce (*Picea abies*). Pine was planted as containerized and bareroot seedlings, but spruce as containerized, bareroot, and bareroot-container hybrid seedlings (Table 1).

Table 1. Planting and tending operations in study sites. Stocktypes include bareroot (BR), bareroot-container hybrid (BRCH), or containerized (C) seedlings

Soil preparation method	Site	Species	Stock-type	Planting: Number of workers monitored	Planting: Number of devices used	Tending: Number of workers monitored		Tending: Number of devices used	
				2017		2017	2018	2017	2018
Mounding	1	Spruce	BR	2	1	-	1	-	1
	2	Spruce	BRCH	3	1	-	2	-	2
	3	Spruce	C	2	1	3	2	3	2
	4	Pine	C	2	1	1	-	1	-
	5	Pine	C	3	1	-	-	-	-
	6	Pine	C	3	1	-	-	-	-
Disc trenching	1	Spruce	BRCH	1	1	-	1	-	1
	2	Spruce	BRCH	1	1	1	1	1	1
	3	Spruce	BRCH	2	1	1	1	1	1
	4	Pine	C	3	1	-	1	-	1
	5	Pine	BR	2	1	-	-	-	-
	6	Pine	C	1	1	-	-	-	-

Table 2. Monitored activities in planting time studies

No.	Activity	Activity description
1.	Planting spot preparation	Planting spot prepared by cleaning away branches, roots, and other logging residues.
2.	Seedling separation/sorting	Seedling separation and sorting before planting activity.
3.	Planting	Planting bareroot seedlings; this activity includes hole digging, planting, and stamping. Planting containerized seedlings, this activity includes seedling insertion in planting tube, planting, and stamping.
4.	Moving in the site	Moving between planting spots.
5.	Going after seedlings	Going after new seedlings, when all picked/carried seedlings from planting basket are planted. Usually 50–100 seedlings are carried with worker in one planting session. Containerized seedlings are more compact and usually in one session 100 seedlings are planted, where usually 50–70 bareroot seedlings are planted in one session. Seedlings usually are stored in edge of planting site and distance to them can greatly vary in different sites. This activity was monitored, but excluded from productivity calculations, because of huge differences between sites.
6.	Other activities	Non-planting activities during planting (talking on the phone, talking to each other, small pauses, etc.).

Time studies for planting were carried out in spring of 2017. Time spent planting one seedling was set as one working cycle. To compare planting productivity on different sites, several planting activities within a working cycle were counted (Table 2). The time study was done using the SDI 1.2. time keeping program (Haglof Sweden AB) that was installed on an Allegro CX field computer (Juniper Systems, USA). During time studies, if possible, data was recorded from several field workers on the site in order to obtain more objective results.

Tending time studies

Time studies for tending were conducted from autumn 2017 until autumn 2018. Tending was implemented on the same sites where planting was done (Table 1). Tending was done on 8 sites, 4 sites were prepared with mounding and 4 sites with disc trenching. On 3 sites, tending was done twice, once in 2017 and once 2018.

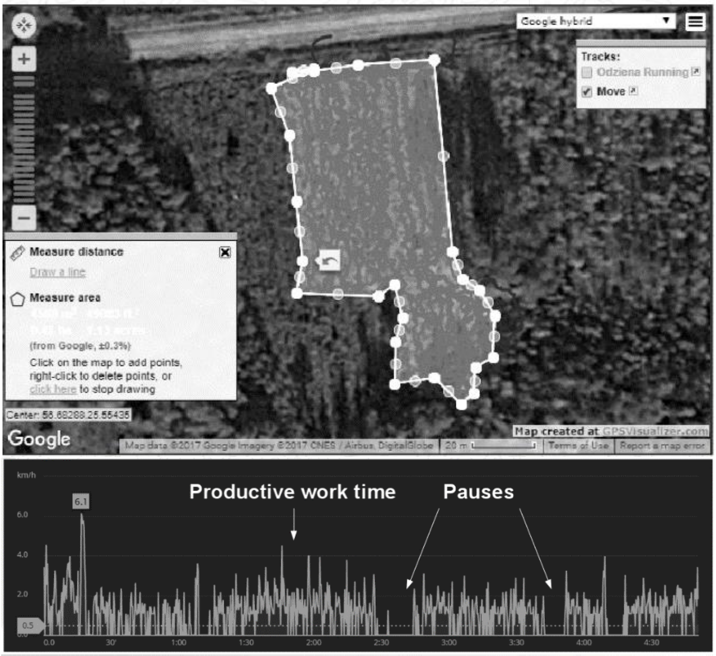
Tending was monitored using GPS devices that were attached to workers. For data recording, simple and freely available GPS sport devices were used to test if these devices could be used in tending time studies. Devices were not selected based on any specific parameters, but were chosen because they were available at this particular moment. We used one unit of each device, and in total 3 different devices were used: Garmin 610 and Garmin F25 (Garmin, Kansas, USA) and Suunto GPS Pod (Suunto, Vantaa, Finland) (Fig. 2).

Figure 2. GPS devices used to quantify tending activities.

If on one site one worker did the tending, a GPS device that was available at this particular day was attached to the worker. If on one site several workers did the tending, available GPS devices were attached to the workers. We did attempt to compare the different devices. During the working time, GPS devices provided non-stop data recordings, where worker walking speed and location was recorded. Tended area was calculated in a 'GPS Visualizer' program, which is free software program available at: http://www.gpsvisualizer.com.

Working time and distance for tending was taken from GPS movement data. (Fig. 3).

Productive work time was considered, when workers were moving and doing tending operation. Pauses included larger breaks, bush saw maintenance, and refueling. In Fig. 3, tending was done in trenches where containerized pine seedlings were planted. Tending was done by one worker and total tended area was 0.46 ha with total time 4 hours and 32 minutes, what converted to one hectare tending time is 9 hours and 51 minute.



Total Area	0,46 ha
Tending time	4:02:00 h
Pauses	0:30:00 h
Total time	4:32:00 h
1 ha	**9:51:00 h**

Figure 3. Working time calculation in tending.

RESULTS AND DISCUSSION

Planting productivity

On average, it took less time to plant container seedlings than bareroot seedlings, regardless of site preparation technique. The average planting time for one bareroot seedling was 23.2 seconds while for containerized seedlings it was 9.8 seconds, which is 57% faster. Containerized seedling planting is faster because of the different seedling root system and planting technique. Containerized seedlings are planted using planting tubes that make planting more effective because main work operations can be done faster than using spades to plant bareroot seedlings. Average bareroot planting spot preparation takes 4.1 seconds, while in containerized seedlings 0.9 seconds, which provides 78% time efficiency. The same applies for the overall planting operation, moving between planting spots and seedling sorting, where time efficiency is 57%, 59% and 18% in favor of containerized seedling planting. Planting with planting tube is physically easier then planting with spade, that is one reason it is easier to maintain steady planting productivity throughout the whole workday, while in bareroot planting the productivity tends to decline in the latter half of working day.

On average, for containers, it took slightly less time to plant mounds than trenches, but for bareroot the result was opposite: planting mounds took much more time than did planting disc trenches. Planting time for one containerized seedling in mounds on different sites varied by 23% and ranged from 7.9 to 10.3 seconds per seedling. The fastest planting was on sites with well-prepared mounds and with moderate ground water level. More variability in planting speed was observed when seedlings were planted in disc trenches, where it varied by 67% and ranged from 4.3 tp 13.9 seconds per seedling.

On average, planting one bareroot seedling in a mound required 28.3 seconds, while in trenches 18.2 seconds per seedling, which is 35% faster. Bareroot seedling planting in mounds was done on one site. Bareroot seedling planting time in trenches on different sites varied by 37% and ranged from 13.5 to 21.4 seconds per seedling. Bareroot planting is slower because it takes more time to find a good planting spot and it takes extra time to clear the for planting, prepare for planting, insert the seedling, and stamp the hole closed (Fig. 4).

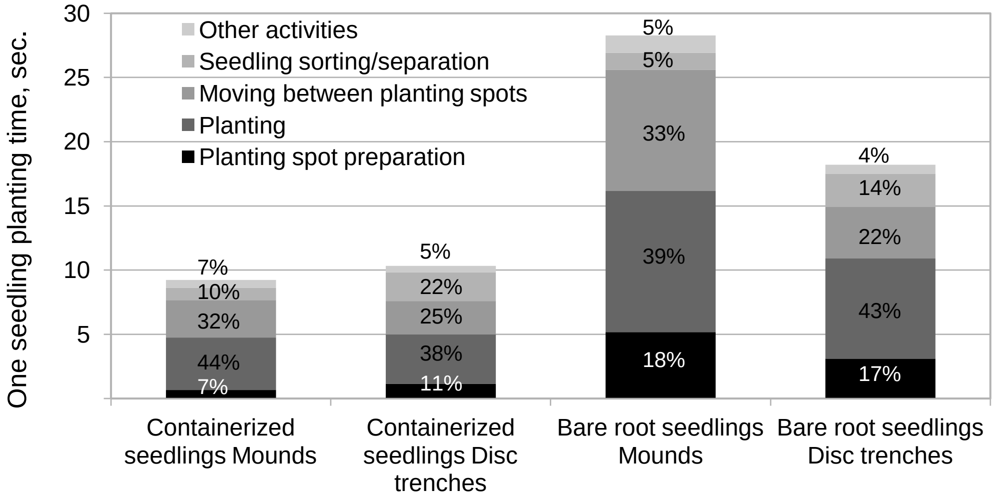


Figure 4. Planting productivity in different soil preparation methods with different coniferous seedlings.

Slower planting occurred on sites with wet trenches and in sites with high logging residues concentration. More time was spent on sites, where soil preparation quality was poor because such sites require extra searching time for proper planting spot and removing logging residues If the soil is prepared properly, no extra time is spent on planting spot preparation. In both soil preparation methods, most of the time was spent on planting operation. Seedling sorting and insertion speed into the planting tube is important when containerized seedlings are planted. Time spent on this activity can be reduced by placing the seedling in the planting tube while moving between planting spots, which leads to a faster planting rate and higher planting productivity in general. Seedling separation is more common in bareroot planting because of root mingling. In containerized seedling planting, this activity mainly is related to seedling withdrawal from plant box and sorting in planting basket.

When planting container seedlings, it was faster to plant spruce at 2,000 per ha than pine at 3,000 per ha. When bareroot-container hybrid spruce seedlings were planted, it was faster to plant seedlings on mounds than in disc trenches. In larger scale (production conditions) planting productivity is measured in hours per hectare. One hectare planting time was calculated from time studies and depends on planted tree species. According to Latvian Forest Law, for pine at least 3,000 and for spruce at least 2,000 seedlings should be planted per hectare to recognize the site restored. Planted tree number is the same for both seedling types: containerized and bareroot. In one hectare planting, time spent for seedling transportation to the site, bringing them into the site, lunch time and other brakes, which are inevitable in planting, were excluded from productivity calculations (Fig. 5).

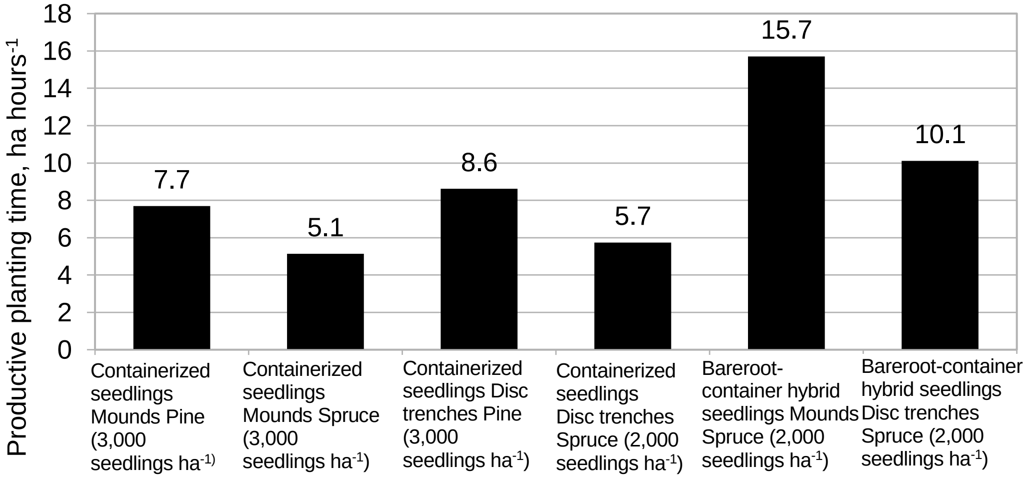


Figure 5. Planting productivity in different soil preparation methods with different tree species.

In this experiment the only bareroot seedlings were spruce seedlings. Although bareroot spruce was planted the most rapidly on disc trenching sites, twice as many container spruce could be planted in the same amount of time. Based on planting time studies, the fastest planting was when containerized spruce seedlings were planted on mounds (5,1 hour per hectare) and the slowest when bareroot spruce seedlings were planted on mounds (15,7 hours per hectare), which is about 60% longer. Previously, time studies for mechanized planting and mounding operation were done in very similar conditions. From these studies mechanized containerized seedling planting on mounds (planting density 2,000 seedlings ha^{-1}) with an M-Planter averaged 11.9 hours per hectare. In other studies (planting density 2,500 seedlings ha^{-1}) with a double-headed M-Planter was 9.6 hours per hectare (Liepins et al., 2011). In mechanized planting, mounding and planting is done at the same time. In our study, when manual planting time is combined with mounding time, average planting productivity for mounding + manual planting was 11.2 hours per hectare (Lazdina et al., 2018).

Manual planting time studies were done in different sites with different soil preparation quality. Crucial factor for fast planting in trenches is water level and logging residues. In wet soils covered with logging residues, planting in trenches is slower due the extra time spent searching for a planting spot and preparation before planting. Planting speed on mounds mostly depends on preparation quality. If the mounds are poorly prepared, it is hard to plant in the middle of the mound and difficult to move between mounds. If mounds are not pressed well so that they have air chambers, planting should be done in the edge of mound but this could cause problems finding seedling during tending.

Tending productivity

Time studies for tending were done to compare tending productivity in mounds and disc trenches. Productive working time, which included only mowing, averaged 81% of the total working time, where pauses, rest breaks, refueling, and maintenance averaged 19% of the total working time. Working time distribution (productive working time vs. pauses, refueling, etc.) with certain exceptions does not change significantly depending on different soil preparation methods (mounds or trenches), planted tree species (pine or spruce), forest type, and level of competition (see. Fig. 6).

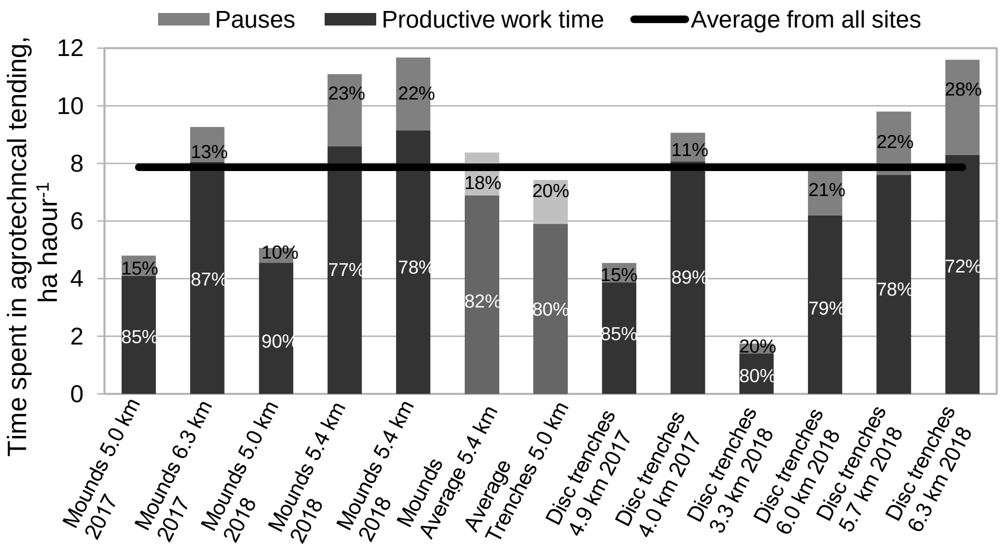


Figure 6. Tending time and walked distance in different soil preparation methods, 2017–2018.

Average time for tending across sites was 7.9 hours per hectare. Time for tending in disc trenches was 1.7–11.6 hours and for mounds 4.8–11.6 hours per hectare. On average, one hectare tending time in mounds was 8.4 hours, while in trenches 7.4 hours, which is 11% faster. These results are similar to those of Dzerina et al. (2016) where tending productivity in mounds was 8.0 hours and in disc trenches 7.7 hours per ha. In Zimelis et al. (2011), where time studies were done in 30 sites and tending was done in trenches, productivity in strips was 5.5 hours per hectare and in continuous (full) tending 7.3 hours per hectare. Previous studies show that seedlings in mounds are protected from surrounding vegetation competition for a longer time period compared to seedlings in trenches (Lehtosalo et al., 2010). Differences in tending productivity depends on the level of competition, worker professionalism, and working organization skills. Overall, tending in trenches is fastest.

Average walked distance for 1 hectare of tending was 5.4 km. Walked distance for tending in mounds was 5.0–6.3 km and for trenches 3.3–6.3 km per hectare. Overall less walking was needed when tending trenches. On average, the walked distance for 1 hectare tending in mounds was 5.4 km but only 5.0 km in trenches, which is 7% shorter. Difference in moving distance depends on worker organizational skills, which allows the worker to choose the shortest distance on a site and plan to exclude walking without mowing.

Tending in trenches is faster because less time is spent deciding on the route and to look where tending has already finished. Workers admit that moving in trenches is easier because they simply follow the trench and moving is mostly on a flat surface. Walking in mounds requires extra attention because of pits between mounds, which could be full with water and logging residues. If the seedlings are not planted in the center of the mound, extra time and attention is spent locating seedlings.

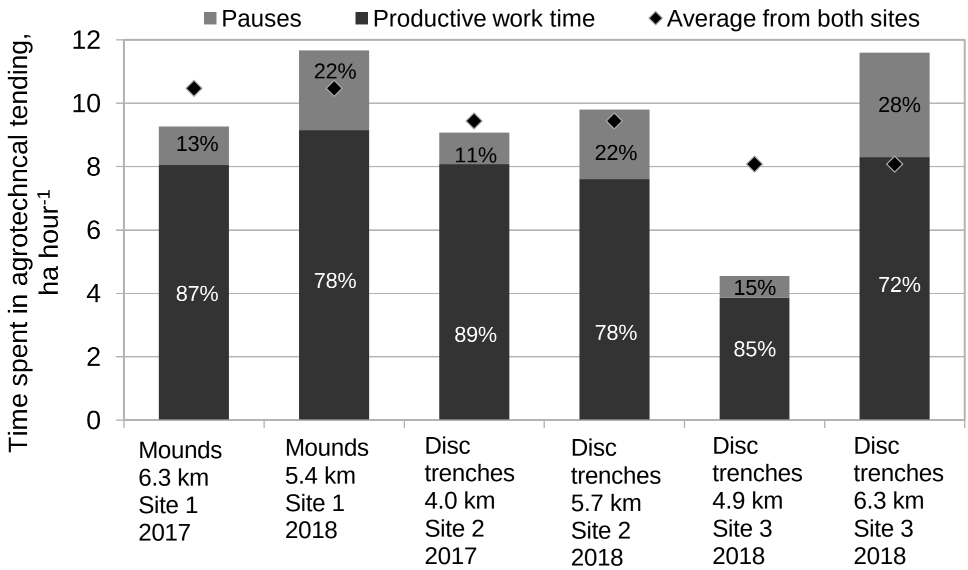


Figure 7. Tending time and walked distance in different soil preparation methods and years.

In 3 sites tending was done twice, in 2017 and 2018 (see. Fig. 7). In all 3 sites more time was spent during the second tending compared to the first one. In all sites, competiton during the first year was smaller compared to second year, and this is the main reason for the drop in tending productivity. Walking distance is not related to tending repetition and is more related to worker professional skills.

CONCLUSIONS

Comparing the productivity of planting speed for containerized and bareroot seedlings, when planting is done in different soil preparation methods (disc trenching and mounding) with different tree species, better results, in terms of productivity, were made by planting containerized seedlings. Comparing containerized seedling planting in different soil preparation methods, better results in terms of productivity were observed when planting on mounds than in trenches. When bareroot planting productivity was compared in different soil preparation methods, planting was more efficient in disc trenches. Workers' professionalism and previous experience is crucial in achieving more productive work. For containerized seedling planting productivity improvement, seedling load in planting tube should be done during the movement between planting spots. Soil preparation quality is important to ensure high planting productivity in both soil preparation methods.

On sites where disc trenches were used, tending productivity was higher and walked distance shorter compared to sites where mounding was used. On sites where tending was done two years in a row, in second year tending took more time, compared to first year.

Our results show that reforestation can be more effective in terms of planting and tending productivity, when species and stocktype are matched to site preparation techniques.

ACKNOWLEDGEMENTS. This study was part of the JSC 'Latvian State Forest' research program 'Forest regeneration, establishment, tending and thinning program 2016–2020'.

REFERENCES

Central Statistical Bureau of Latvia. 2018. Forestry in 2017. Available at https://www.zm.gov.lv/public/ck/files/skaitlifakti_EN_2018web.pdf (in Latvian).

Dzerina, B., Girdziusas, S., Lazdina, D., Lazdins, A., Jansons, J., Neimane, U. & Jansons, Ā. 2016. Influence of spot mounding on height growth and tending of Norway spruce: case study in Latvia. *Forestry Studies/Metsanduslikud Uurimused* **65**, 24–33.

Heiskanen, J., Saksa, T. & Luoranen, J. 2013. Soil preparation method affects outplanting success of Norway spruce container seedlings on till soils susceptible to frost heave. *Silva Fennica* **47**(1) article id 893, 1–17.

Jansons, Ā., Donis, J., Danusevičius, D. & Baumanis, I. 2015. Differential analysis for next breeding cycle for Norway spruce in Latvia. *Baltic Forestry* **21**(2), 285–297.

Latvian State Forests 2015. Planting, sowing and replenishment instructions. Available at https://www.mezsaimnieks.lv/images/userfiles/stadi_final_WEB3.pdf (in Latvian)

Law on forest. 2000. *Latvijas Vestnesis* 98/99 (2009/2010), 16.03.2000., *Zinotajs*, 8, 20.04.2000. Available at: https://likumi.lv/ta/en/en/id/2825

Lazdina, D. 2017. The forest regeneration, establishment and cleaning research program. Overview of the results Available at: http://www.silava.lv/userfiles/file/Projektu%20parskati/2017_Lazdina_LVM_MAIKP_www.pdf. (in Latvian)

Lazdina, D., Makovskis, K., Stals, T.A., Dumins, K. & Lazdins, A. 2018. Mounding and mechanized planting in forest regeneration in changing climate conditions. 51st. *International Symposium of forest mechanization, FORMEC 2018 – Improved Forest Mechanisation: mobilizing natural resources and preventing wildfires*, 24 – 28 September, Madrid, Spain. 245–253. https://www.formec.org/images/proceedings/2018/Proceedings2018.PDF

Lazdins, A., Kaleja, S., Gruduls, K. & Bardulis, A. 2013. *Research For Rural Development* **2**, 42–48.

Lazdins, A., Prindulis, U., Kaleja, S., Daugaviete, M. & Zimelis, A. 2016. Productivity of Vimek 404 T5 harvester and Vimek 610 forwarder in early thinning. *Agronomy Research* **14**(2), 475–484.

Lehtosalo, M., Mäkelä, A. & Valkonen, S. 2010. Regeneration and tree growth dynamics of *Picea abies*, *Betula pendula* and *Betula pubescens* in regeneration areas treated with spot mounding in southern Finland. *Scandinavian Journal of Forest Research* **5**, 213–223.

Liepins, K., Lazdina, D. & Lazdins, A. 2011. Productivity and cost-effectiveness of the M-planter tree planting machine in Latvian Conditions. *Baltic Forestry* **17**(2), 308–311.

Nordborg, F., Nilsson, U. & Örlander, G. 2003. Effects of different soil treatments on growth and net nitrogen uptake of newly planted (L.) Karst. seedlings. *Forest Ecology and Management* **180**, 571–582.

Petaja, G., Kaleja, S., Zimelis, A. & Lazdins, A. 2018. Comparison of productivity of standard and accumulating forwarder grapple in thinning. *Engineering for Rural Development* **17**, 1366–1371.

UOT-2000 Forest trencher. 2019. Available at: http://www.uotforest.com/production/uot-2000-forest-trencher/

Uotila, K., Rantala, J. & Saksa, T. 2012. Estimating the need for early cleaning in Norway spruce plantations in Finland. *Silva Fennica* **46**(5), 683–693.

Uotila, K., Rantala, J., Saksa, T. & Harstela, P. 2010. Effect of soil preparation method on economic result of Norway spruce regeneration chain. *Silva Fennica* **44**(3), 511–524

Uotila, K. & Saksa, T. 2014. Effects of early cleaning on young *Picea abies* stands *Scandinavian Journal of Forest Research* **29**(2), 111–119.

Vyse, A.H. 1973. Applying work study techniques to tree planting. *Tree Planters' Notes* **24**(3), 19–23.

Zālītis, P., Lībiete, Z. & Jansons, J. 2017. Kokaudžu augšana mūsdienīgi veidotās jaunaudzēs. Salaspils: LVMI Silava, DU AA "Saule", 117 pp.

Zimelis, A., Lazdina, D., Donis, J., Lazdāns, V. & Grīnvalds, A. 2011. Work productivity and cost analysis for forest thinning and tending. *Study overview*, pp. 1–58. Available at http://www.silava.lv/userfiles/file/Projektu%20parskati/2011_Zimelis_LVM.pdf (in Latvian)

Agronomy Research **17**(6), 2339–2349, 2019
https://doi.org/10.15159/AR.19.199

Biological effect of hydroxycitric acid within a *Garcinia Cambogia* extract on the nutrient metabolism

V.V. Martirosyan[1,3], Kh.A. Baluyan[1], M.N. Kostyuchenko[1], V.D. Malkina[2], E.V. Zhirkova[4] and M.F. Marshalkin[5]

[1]Research Institute of Baking Industry, st. B. Cherkizovskaya, d. 26 ‘a’, RU107553 Moscow, Russia
[2]Moscow State University of Technology and Management named after K.G. Razumovsky (PKU), st. Ground Val, 73, RU109004 Moscow, Russia
[3]All-Russian Research Institute of Corn, st. Yermolov, 14 ‘b’, RU357528 Pyatigorsk, Russia
[4]Russian Economic University named after G.V. Plekhanov, Stremyanny Lane, 36, RU117997 Moscow, Russia
[5]North-Caucasian Federal University, Pyatigorsk Branch, 40 Let Octyabrya Ave. 56, RU357500 Pyatigorsk, Russia
[*]Correspondence: v.martirosyan@gosniihp.ru

Abstract. Among plant supplements, which once included in a food regimen, induce a favorable evolution in persons seeking to lose some weight, *Garcinia cambogia* fruit containing hydroxycitric acid deserves close attention. The aim of this study consisted in the investigation of a biological effect of hydroxycitric acid within a *Garcinia cambogia* extract on the nutrient metabolism. The study involved the use of a *Garcinia cambogia* extract comprising hyroxycitric acid in the amount of 60.23%. In view of simulating the conditions of digestion, model dietary media consisting of extrusion products added with a *Garcinia cambogia* extract and enzymes were developed. The simulation of digestion processes in experimental model media has shown the decrease of glucose formation. Possibly hydroxycitric acid inactivates the activity of amylases, what results in the decline in the content of free monosaccharides and in the reduction of synthesis of glycerol as a fat component with the effect of an inhibited triglyceride formation. The addition of an extrusion product comprising the *Garcinia cambogia* extract to the diet of laboratory animals (rats) after 28 days of the experiment resulted in a reliable reduction of blood total cholesterol and triglycerides by 13% and 28%, respectively, and also in the decrease of body weight of animals by 5.8%. The investigation confirms the data available in the literature on the properties of hydroxycitric acid within a *Garcinia cambogia* extract, which influences the nutrient metabolism, thereby allows using brindleberry processing products for the correction of body weight.

Key words: *Garcinia cambogia*, extrusion product, fats, hydroxycitric acid, metabolism, overweight.

INTRODUCTION

In the recent years the nutrition structure of contemporary population has undergone negative changes: the increase of the share of refined food products, the incorporation in the diet of semi-finished products and fast food with a high content of easily digested carbohydrates and trans-isomers of fatty acids. Said factors contribute to the excess of dietary calories over energy expenditure resulting in the growth of overweight and obesity.

According to the global estimation of the World Health Organization in 2016 over 1.9 billion adults over 18 had overweight and over 650 million of these had obesity. From 1975 to 2016 the number of obese people in the world has more than tripled (WHO, 2018).

There is a similar trend in Russia, according to the data of the Federal Research Center of Nutrition and Biotechnology about 60% of women and 50% of men over 30 have overweight, about 26% of Russian people have obesity. Notably that the number of overweight population is continuously increasing: in 2005 the percentage of obese people was 23% and in 2012 was 25.3% (Basharova, 2016).

An overweight and obesity result in the reduction of life expectancy and the decrease of the quality of life, they are the principal risk factors of non-contagious diseases: cardiovascular diseases (heart diseases, stroke, ischemia); diabetes mellitus; musculoskeletal disorders; some cancers (Shutova & Danilova, 2004).

At present there are various methods of weight loss: drug treatment, surgical interference, diet therapy. The most common and preferred method consists in the development of a balanced diet based on the consumption of healthy foods. With the aim of maintaining the overall health, the food industry produces products enriched with vitamin and mineral complexes, dietary fibers, plant extracts and other functional supplements (Sadovoy et al., 2012).

In view of the foregoing, for solving the global problem responsible for the increase of the body weight it is necessary to search for components inhibiting the lipogenesis in an organism and investigate the mechanism of their action. Among plant supplements, which once included in a food regimen, induce a favorable evolution in persons seeking to lose some weight, *Garcinia cambogia* fruit deserves close attention.

Studies of many researchers are related to the investigation of a mechanism of the effect of *Garcinia cambogia* extract on metabolic processes in an organism. Yimam et al. (2019) carried out their study on laboratory animals and determined that a *Garcinia cambogia* extract promoted the suppression of appetite. Downs et al. (2005) has come to a similar conclusion, while determining experimentally that a complex compound of hydroxycitric acid contained in a *Garcinia cambogia* extract with calcium and potassium salts (dietary supplement HCA-SX) enhances the effect of the extract and results in the suppression of appetite and the decrease of the body weight, what is due to the increased availability of serotonin and the declined blood lipid content. Semwal et al. (2015) have determined that substances comprised in a *Garcinia cambogia* extract influence the level of serotonin which is able to enhance gastrointestinal peristalsis and secretory activity what results in a decreased food consumption. The paper of Márquez et al. (2012) presents the results of investigation of the efficiency of a *Garcinia cambogia* extract in the regulation of endogenous lipid biosynthesis. The authors explain the effect by the fact that hydroxycitric acid inhibits the citrate lyase enzyme, which is directly involved in lipid formation processes. A study was related to the effect of hydroxycitric acid on the decrease of body weight of broiler chicken. Han et al. (2016) have determined that the incorporation of hydroxycitric acid in the amount of 3,000 mg kg^{-1} in a diet of broiler chicken inhibited the expression of SREBP-1C protein binding a fatty acid synthase. The study of Raina et al. (2016) has determined that in addition to the effect of reducing the body weight a *Garcinia cambogia* extract has anti-inflammatory, antiulcerogenic, antioxidant and hepatoprotection effects. Moreover the papers (Koshy et al., 2001; Hayamizu et al., 2003a and 2003b; Tharachand et al., 2015) also confirm biological properties of a *Garcinia cambogia* extract promoting the decline of the body weight.

Consequently there is no consensus in the literature on a mechanism of action of biologically active substances contained in a *Garcinia cambogia* extract on the nutrient metabolism of an organism resulting in the decrease of body weight.

The aim of this study consists in the investigation of a biological effect of hydroxycitric acid (HCA) within a *Garcinia cambogia* extract (GCE) on the nutrient metabolism.

MATERIALS AND METHODS

The research involved the use of a GCE (manufactured by Eusa Colors Sas, France). Physical and chemical quality parameters are provided in the batch certificate (GCE - 121017): color – white; taste and odor – specific for the extract; weight percentage of hydroxycitric acid – 60.23%; moistue content – 3.63%; ash content – 3.78%. The GCE was incorporated into extrusion products to achieve its uniform distribution in ready products and dietary media. For a uniform distribution of the GCE in dietary media it was introduced into a maize grit extrudate of Beshtau maize hybrid produced in the All-Russia Research Institute of Maize. The use of Beshtau maize hybrid grit is due to a predominant content in the grain of a high amylase starch acting as a neutral carrier for the GCE components. The extrusion treatment was performed with the use of a single screw laboratory extruder under the following conditions: temperature 140–145° C, moisture of raw material 16–18%, screw speed 160 rpm, screw length 40 cm, screw diameter 38 mm, die diameter 5 mm, pressure in the pre-die chamber of the extruder 5.5 to 6.2 MPa. The study was designed to use an extrusion product containing the GCE in the amount of 7% relative to the grit weigh (Malkina & Baluyan, 2016).

The content of monosaccharides in dietary media was determined by the ion chromatography with the Thermo Scientific Dionex ICS 5000+ (column CarboPac PA20 3*150, protective column Amino Trap 4*50). A 1 g sample of the extrusion product was added with 10 mL of deionized water, carefully mixed until the complete dissolution, added with 5 mL of trichloroacetic acid to precipitate protein substances, centrifugated for 20 min and subsequently filtered. The obtained filtrate was diluted tenfold and the samples were used for the analysis.

The fatty acid composition of dietary media was determined by the gas-liquid chromatography with the use of the instrument HP 4890 with a flame ionization detector. The operating parameters of the instrument: evaporator temperature: 240° C; detector temperature: 270° C, fused silica capillary column HP-INNOWAX, 30 m×0.25 mm, film thickness: 0.25 μm, carrier gas: nitrogen, carrier gas flow rate: 1.5 cm^3 min^{-1}. Programming mode of the temperature of the column thermostat is from 50° C to 240° C at the rate of 10° C per minute.

The content of hydroxycitric acid in the extrusion products was determined by the high performance liquid chromatography with 'Agilent 1100' liquid chromatograph provided with a 'VWR' detector. Organic acids were separated in a chromatographic column filled with octadecyl silica gel. The calibration curve was built in coordinates S (peak response) – C (acid concentration), g dm^{-3} within the concentration range of 0.1 to 40 g dm^{-3}. The concentration of hydroxycitric acid was calculated from chromatographic peak responses obtained with the use of a spectrophotometric detector at $\lambda = 210$ nm (Wavelength = 210 nm) according to the external standard method.

The biological effect of the GCE on nutrient metabolism was evaluated on laboratory animals in conditions of a fundamental research laboratory for experimental immunology, immunopathology and immunobiotechnology of the North-Caucasian Federal University. The experiment was performed on Wistar male rats of 280–340 g. Experimental tests with laboratory rats were carried out in accordance with the generally accepted ethical standards for animal experimentation in compliance with the rules adopted by the European Convention for the Protection of Vertebrate Animals Used for Experimental and other Scientific Purposes (1986).

During the experiment the animals were kept in standard conditions of a laboratory vivarium in similar care, nutrition, light and temperature conditions with a free access to water and feed. With the purpose of adaptation, the animals were kept in cages in groups for 14 days prior to the study and received equicaloric diet. During this period their physical condition was evaluated every day by a visual examination.

The experiment included the preparation of pills containing the extrusion product. To this end the grinded extrusion product comprising the GCE was mixed with the same amount of flour in all cases. The pills were prepared every day directly prior to feeding the animals. The prepared pills were given once a day 2 hours before the basic diet was placed in the cages. Pill eating by the animals was monitored. Blood samples were collected from the tail vein of the rats with a subsequent serum separation in a bench-top centrifuge MicroCL 17R (Thermo) at 3,000 rpm for 15 min. Total cholesterol and triglycerides in the blood serum were determined with a biochemical semi-automated analyzer BioChem SA Plus (HTI) with the use of reagent kits manufactured by High Technology (USA). The obtained results were statistically processed using the Student's test. The samples were analyzed in triplicate. The calculations were performed with the use of Biostat software (version 4.03).

RESULTS AND DISCUSSION

The preparation of the extrusion product included a coextrusion of Beshtau maize hybrid grit and the GCE in an amount of 7% of the grit weight. During the extrusion processing a plant raw material is exposed to physical factors such as a high temperature, pressure, mechanical stress. Physical factors may result in the neutralization of biological activity of the GCE component. In view of this, the preservation rate of hydroxycitric acid in grain extrusion products was determined by the HPLC with the use of a liquid chromatograph 'Agilent 1100' provided with a VWR detector.

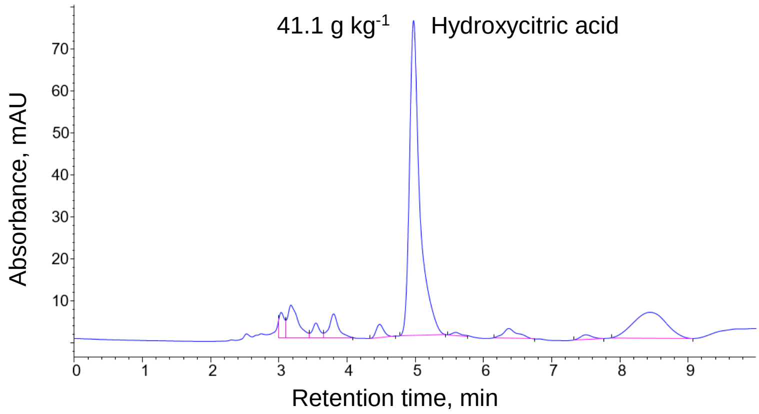


Figure 1. HCA content in the extrusion product containing the GCE.

The extrusion product added with 7% of the GCE was shown to contain hydroxycitric acid in the amount of 41.1 g kg^{-1}. The HCA preservation rate (90%) determined experimentally following the coextrusion with the high-amylose maize grain is due to the HCA insulation from external influences by its incorporation into the matrix structure of gelatinized starch.

For purposes of investigation of possible nutrient metabolism variants, model dietary media were prepared with the use of the extrusion product and enzymes (Table 1).

Table 1. Variants of model dietary media

Sample number	Model dietary media added with enzymes, 0.002% relative to the weight of the extrusion product			
	amylase	lipase	trypsin	amylase + lipase + trypsin
Extrusion product (control)				
1	+			
2		+		
3			+	
4				+
Extrusion product added with the GCE, 7% (experiment)				
5	+			
6		+		
7			+	
8				+

Samples of the extrusion products were grinded, added with an amount of water necessary to achieve the moisture content of 50% and placed to an incubator at the temperature of 37° C. In samples 1 and 5 the pH was 6.8–7.0 with the incubation time of 60 minutes, for the amylase activity is maximal in a slightly alkaline medium. For ensuring the optimal effect of trypsin (samples 3 and 7) a slightly alkaline medium was also generated with the pH of 7.8–8.0 and the time of incubation was 120 minutes. Samples 2 and 6 were incubated for 180 minutes at pH of 7.6–7.8 to provide an active effect of lipase on a respective substrate. For samples 4 and 8, where the exposure to all three enzymes has to be ensured, optimal conditions for each enzyme were created gradually. Once the model dietary media were incubated, the amount of monosaccharides and a fatty acid composition of the media were determined.

Fig. 2 shows amounts of monosaccharides (fructose, arabinose, galactose, glucose) detected in the model dietary media. The data evidence that with the addition of amylase to a dietary medium polysaccharides are subjected to an enzymatic hydrolysis with the production of glucose. In the experiment with the addition of amylase to the extrusion product containing the GCE (sample 5) the amount of resulting glucose was decreased by 30% in comparison with the extrusion product also containing the amylolytic enzyme but without addition of the GCE (sample 1). When the enzyme complex was used with the extrusion product added with the GCE (sample 8) the decline of the production of glucose by 8% was also recorded in comparison with sample 4. In our opinion, this is explained by the ability of hydroxycitric acid to create conditions for inactivation of amylolytic enzymes, what results in the decline of concentration of monosaccharides involved in fat synthesis (Marshalkin, 2016). The monosaccharides – glucose, fructose, galactose – formed in the hydrolysis of complex carbohydrates can be interconverted under the effect of specific enzymes (Filippovich, 1998).

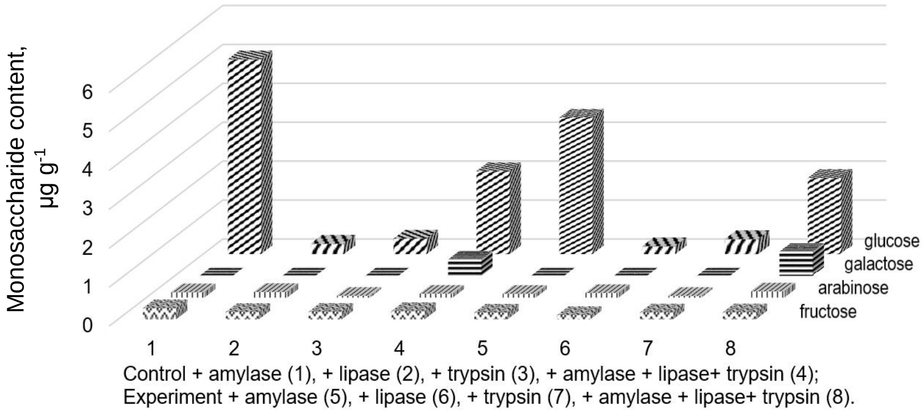
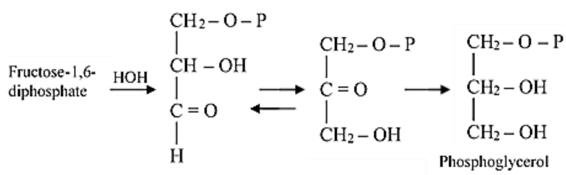


Figure 2. Monosaccharide content in model dietary media.

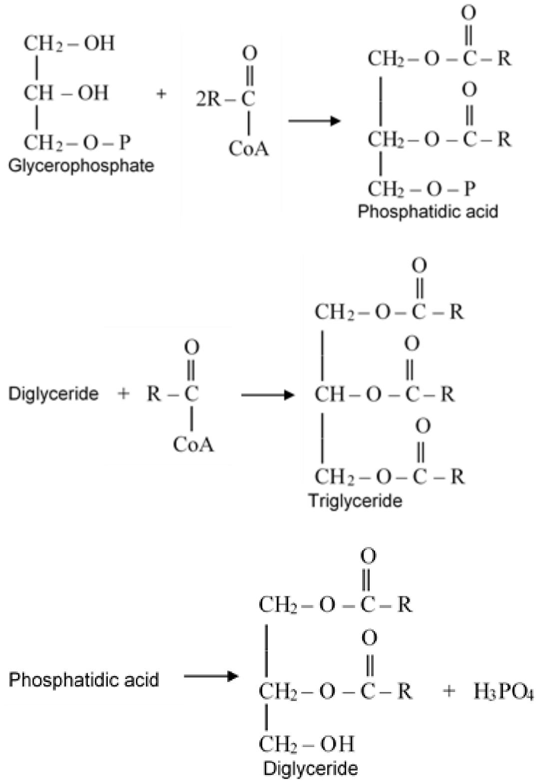
As follows from the reactions of fat synthesis (Marshalkin, 2016, Bruhman, 1981), the amount of carbohydrates is a crucial factor in the process of forming fats – triglycerides. Their synthesis requires glycerol and fatty acids. Given that neutral lipids are glycerol esters with higher fatty acids, the biosynthesis of both components: glycerol and higher fatty acids should be considered individually.

Glycerol is formed from monosaccharides via the known pathway (Bruhman, 1981), where fructose is used as a substrate for the production of one of the fat components such as glycerol. Under the effect of the enzyme aldolase fructose-1,6-diphosphate breaks down to phosphoglyceraldehyde and phosphodioxyacetone which is easily enzymatically reduced to phosphoglycerol. The formed phosphorylated glycerol directly participates in fat synthesis.



As provided above (Fig. 2) dietary media containing the GCE had a lower carbohydrate content in relation to the samples containing no HCA, what according to the theory of glycerol formation from carbohydrates would result in a reduced glycerol concentration in dietary media.

The biosynthesis of neutral fats takes place in microsomes in the presence of glycerol in the form of glycerophosphate and activated fatty acids. The interaction of active fatty acids and glycerophosphate proceeds stepwise. First two fatty acid residues are attached to glycerol to form phosphatidic acid. Then phosphatidic acid loses the phosphate residue under the effect of the phosphatase enzyme is converted into a diglyceride (Marshalkin, 2016; Bruhman, 1981). Finally, the third active fatty acid molecule is attached to the diglyceride to form a triglyceride.



The neutral fat so synthesized is used in the organism for various purposes, while its excessive portion is deposited in fat depots (Marshalkin, 2016).

Consequently, the provided scheme of fat synthesis from carbohydrates demonstrates that in the case the content of monosaccharides in dietary media is reduced, glycerol synthesis is inhibited, what in its turn results in the decrease of fat concentration.

A fatty acid composition in model dietary media was investigated by a gas-liquid chromatography, the results of the study are presented in Table 2.

Table 2. Fatty acid composition of model dietary media, % to the sum of fatty acids

Fatty acid	Designation	Extrusion product added with the GCE, 7%			
		Without enzymes	Added with enzymes, 0.002% of the weight of the extrusion product		
			Amylase	Lipase	Trypsin
Myristic	14:00	1.9	1	0.9	1.4
Palmitic	16:00	25	16.8	18.8	16.6
Palmitoleic	16:01	2.1	3.7	1.9	1.9
Stearic	18:00	30.6	8.4	13.9	3.7
Oleic	18:01	10.5	25.5	30.1	30.5
Linoleic	18:02	11.2	31.1	30.7	42.3
Linolenic	18:03	0.6	0.5	0.8	0.5
Arachidonic	20:00	0.5	1.4	1.2	1.7
Content of PUFAs		24.4	60.8	63.5	75.2
Content of UFA		58	27.6	34.8	23.4
Total fatty acids		82.4	88.4	98.3	98.6
Fat, %		0.86	0.82	0.79	0.82

One can see from the data presented in Table 2 that with a combined addition of the GCE and enzymes to a dietary medium the content of polyunsaturated fatty acids is increased. This is a direct evidence of an enhanced degradation of deposited fat under the effect of HCA having an additional hydrophilic oxy-group what contributes to a better solubility of the substrate and hence to the increased velocity of fat hydrolysis reactions.

For confirming the biological effect of HCA within the GCE on the nutrient metabolism the study analyzed the influence of the extrusion product containing 7% of the GCE on blood biochemical indices and changes of the body weight of laboratory animals (male rats) (Malkina et al., 2016).

The animals were divided into 4 groups, six rats in each:

Group I – animals receiving a standard diet (intact control);

Group II – animals receiving a standard diet + an extrusion product containing 7% of the GCE;

Group III – animals receiving a standard diet + an extrusion product without addition of the GCE;

Group IV – animals receiving a standard diet + the GCE.

The animals were fed with prepared pills containing the extrusion product and wheat flour. When an amount of the extrusion product in the pills was selected, the content of the GCE was taken into account. According to the available data, 1,000 mg of the GCE per day was taken as a human therapeutic dose. The human dose was converted into an animal dose in accordance with the manual for experimental (preclinical) study of new pharmacological substances with the use of a conversion factor. Intact control animals (Group I) were only added a flour pill base to the diet. Animals of Group II received once 1,137 mg kg^{-1} of the extrusion product added with the GCE. Animals of Group III received 1,137 mg kg^{-1} of the extrusion product, which contained no GCE. Animals of Group IV received 79.6 mg kg^{-1} of the *Garcinia cambogia* extract (Khabriev, 2005).

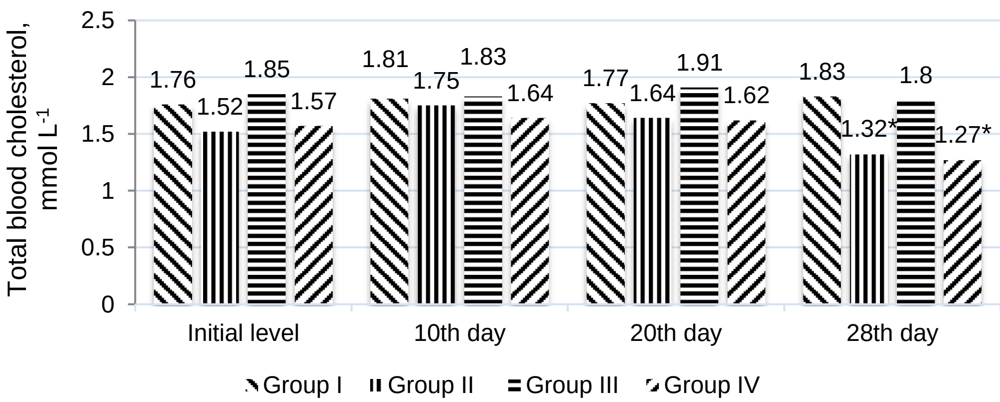


Figure 3. Dynamics of total blood cholesterol.

* – statistically significant changes compared to the initial level at $P < 0.05$.

The data of the experiments showed (Fig. 3) that in comparison with the group of intact control animals (Group I), where no statistically significant fluctuations of lipid spectrum indices was recorded during the experiment, there was a number of changes in the experimental groups.

At the 28th day of the experiment animals of Group IV and Group II demonstrated a reliable decline of total cholesterol respectively by 15% (P < 0.05) and 13% (P < 0.05) compared to the initial level.

The detected decrease of triglycerides in the blood of rats of Group IV on the 20th and the 28th day of the experiment amounted to 27% (P < 0.001) and 23% (P < 0.05), respectively. In the blood of rats of Group II the decrease of triglycerides was recorded on the 28th day of the experiment (28%, P < 0.05) (Fig. 4).

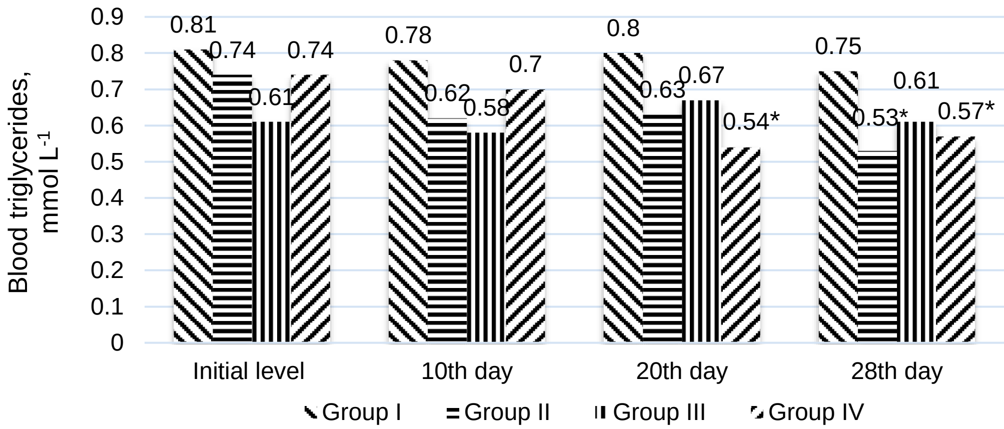


Figure 4. Dynamics of blood triglycerides.

* – statistically significant changes compared to the initial level at P < 0.05.

The average blood indices of total cholesterol and triglycerides in animals of Group III and Group I showed no statistically significant changes during the whole experiment.

During the experimental period animals of Group I and Group III showed no significant changes in body weight mass, the increase was 2.3% and 1.1%, respectively. Considering that there is a modest tendency for increased body weight, we can suppose that it is possibly due to natural growth processes of the animals. Animals od Goup II and Group IV were characterized by a tendency for decreased body weight by 5.8% and 5.7% at the 28th day of the experiment, respectively. The experiments proved that the addition of the GCE (containing HCA) to an animal diet both within extrusion products and in native form resulted in the decrease of the body weight, what correlated with the blood tricglyceride and cholesterol content.

The obtained results characterize the manifestation of biological action of HCA within the GCE, which is expressed in a reliable decrease of cholesterol and triglyceride levels in the blood of animals after the *in vivo* experiment.

CONCLUSIONS

The performed research allows concluding that the mechanism of the GCE action comes down to the manifestation of at least two biological effects under the influence of hydroxycitric acid (the principal biologically active component of the GCE) on the nutrient metabolism in an organism:

– the inhibition of fat synthesis from carbohydrates determined by the inactivation of amylolytic enzymes;

– the promotion of the degradation of fats including these from depots, what is evidenced by the increased amount of free fatty acids in dietary media containing the GCE and also a reliable decrease of total cholesterol and triglycerides in the blood of animals.

The use of an extrusion product containing the GCE in *in vivo* experiments resulted in a reliable decline of blood total cholesterol and triglycerides and also in a reduction of body weight of animals, what supports the data available in the literature on *Garcinia cambogia* properties facilitating the correction of the body weight.

The performed investigation of the biological action of hydroxycitric acid within the *Garcinia cambogia* extract is a theoretical substantiation of its application in food technologies as a biologically active ingredient for correcting the body weight, what will promote the health of the population.

ACKNOWLEDGEMENTS. The article was prepared in the framework of the research grant MD - 4862.2016.11.

REFERENCES

Basharova, S. 2016. Every fifth Russian suffers from obesity. *Izvestiya 14 January*. Moscow, pp. 3–4 (in Russian).

Bruhman, E. 1981. Applied Biochemistry: translated from German. *Legkaya i pishchevaya promyshlennost*, Moscow, 296 pp. (in Russian).

Downs, B., Bagchi, M., Subbaraju, G., Shara, M., Preuss, H. & Bagchi, D. 2005. Bioefficacy of a novel calcium–potassium salt of -hydroxycitric acid. *Mutation research/fundamental and molecular mechanisms of mutagenesis* **1–2**, 149–162.

European Convention for the Protection of Vertebrate Animals Used for Experimental and other Scientific Purposes (ETS 123). 1986. Strasbourg, 11 pp.

Filippovich, Yu. 1998. Fundamentals of Biochemistry. In: *4th edition, revised*. 'Agar' Editorial House. Moscow, 512 pp. (in Russian).

Han, J., Li, L., Wang, D. & Ma, H. 2016. Hydroxycitric acid reduced fat deposition via regulating lipid metabolism-related gene expression in broiler chickens. *Lipids Health Dis* **15**, 37–50.

Hayamizu, K., Hirakawa, H., Oikawa, D., Nakanishi, T., Takagi, T., Tachibana, T. & Furuse, M. 2003a. Effect of Garcinia cambogia extract on serum leptin and insulin in mice. *Fitoterapia* **74**, 267–273.

Hayamizu, K., Ishii, Y., Kaneko, I., Shen, M., Okuhara, Y., Shigematsu, N., Tomi, H., Furuse, M., Yoshino, G. & Shimasaki, H. 2003b. Effects of Garcinia cambogia (Hydroxycitric Acid) on visceral fat accumulation: a double-blind, randomized, placebo-controlled trial. *Current therapeutic research* **8**, 551–567.

Khabriev, R. 2005. Manual for experimental (preclinical) study of new pharmaceutical agents. *Meditsina*, Moscow, 832 pp. (in Russian).

Koshy, A., Anila, L. & Vijayalakshmi, A. 2001. Flavonoids from Garcinia cambogia lower lipid levels in hypercholesterolemic rats. *Food Chemistry* **3**, 289–294.

Malkina, V., Baluyan, Kh., Zhirkova, E., Piskov, S. & Martirosyan, V. 2016. Grain product for dietary nutrition. *Konditerskoe i khlebopekarnoe proizvodstvo* **5–6**, 31–33 (in Russian).

Márquez, F., Babio, N., Bulló, M. & Salas-Salvadó, J. 2012. Evaluation of the safety and efficacy of hydroxycitric acid or garcinia cambogia extracts in humans. *Critical reviews in food science and nutrition* **7**, 585–594.

Marshalkin, M. 2016. Biochemistry: study guide. *Institute of Service, Tourism and Design (branch) of the North-Caucasian Federal University in Pyatigorsk*, Moscow, 323 pp. (in Russian).

News Bulletin ‘Obesity and overweight’, World Health Organization 2018. *https://www.who.int/ru/news-room/fact-sheets/detail/obesity-and-overweight.* Accessed 17.2.2018. (in Russian).

Raina, R., Mondhe, M., Malik, K. & Gupta, C. 2016. Garcinia cambogia. *Nutraceuticals: Efficacy, Safety and Toxicity*, 669–680.

Sadovoy, V., Aralina, A., Bunina, O. & Shchedrina, T. 2012. Evaluation of the efficiency of biologically active supplements for preventing obesity. *Izvestiya VUZov. Pishchevaya tekhnologiya* **5–6**, pp. 96–98 (in Russian).

Semwal, R., Semwal, D., Vermaak, I. & Viljoen, A. 2015. A comprehensive scientific overview of Garcinia cambogia. *Fitoterapia* **102**, 134–148.

Shutova, V. & Danilova, L. 2004. Obesity or overweight syndrome. *Meditsinskie novosti* **7**, pp. 10–12 (in Russian).

Tharachand, C., Immanuel Selvaraj, C. & Abraham, Z. 2015. Comparative evaluation of anthelmintic and antibacterial activities in leaves and fruits of garcinia cambogia (gaertn.) desr. and garcinia indica (dupetit-thouars) choisy. *Braz. Arch. Biol. Technol* **3**, 379–386.

Yimam, M., Jiao, P., Hong, M., Brownell, L., Lee, Y., Hyun, E., Kim, J., Nam, J., Kim, M. & Jia, Q. 2019. Evaluation of natural product compositions for appetite suppression. *Journal of dietary supplements* **16**, 86–104.

Agronomy Research **17**(6), 2350–2358, 2019
https://doi.org/10.15159/AR.19.202

Influence of precipitation and moisture reserves on the yield of crops under different tillage

G. Mokrikov, T. Minnikova*, K. Kazeev and S. Kolesnikov

Southern Federal University, Academy of Biology and Biotechnology by D.I. Ivanovsky's, Department of Ecology and Nature Management, av. Stachki 194/1, RU344090 Rostov on Done, Russia
*Correspondence: loko261008@yandex.ru

Abstract. Tillage technologies that promote resource-saving and increase in the yield of agricultural crops are being increasingly involved into the agriculture of arid territories of Russian Federation. Studies of the impact of new tillage on soil quality and yield in Russian Federation are necessary owing to the high soils and climate diversity. Yield enhancement of major crops - winter wheat and sunflower - have been observed in Russian Federation in recent years. During 2014–2019 in the south of the European part of Russian Federation (Rostov region), the effect of No-Till (NT) on soil quality and yield of field crops was studied. The studies were carried out over an area of 5,500 hectares in comparison with adjacent fields, where conventional tillage (CT) of soils with mould board plowing was used. The yield of sunflower and winter wheat depended significantly on the amount of precipitation during the growing season. In 2014–2017 the use of No-Till increased the yield of winter wheat by 26–114%, of sunflower - by 27–92% as compared with farms, where the conventional tillage of soil treatment was used. No-Till helped to save motor fuel, increase yields of agricultural crops and lower the cost of winter wheat and sunflower.

Key words: conventional tillage, crop yield, fertility, ordinary chernozem, no-till, sunflower, winter wheat.

INTRODUCTION

The population of the Earth increases yearly by an average of 1.00–1.22% (up to 100.0–122.2 million people). Regular increase in population size compels people to look for new food sources and intensively develop new agricultural land or the use of existing ones. Along with the need to develop the agricultural sector, the question of the rational use of non-renewable energy sources such as oil and gas arises. In recent years, Russian Federation has been actively discussing the possibility of saving motor fuel in agriculture without affecting the yield of agricultural crops and soil fertility. Modern tillage technologies include a set of measures for the mechanical and chemical tillage: mould board plowing, fertilizer application and pesticide treatment, etc. Different tillage techniques change the agrophysical properties of the soil (Ivelic-Sáez et al., 2015; Zúñiga et al., 2015; Gailis et al., 2017; Bai et al., 2018; Dridiger et al., 2018; Zelensky et al., 2018; Trofimova et al., 2018). The most significant is a change in soil structure and a decrease in soil permeability compared with conventional soil treatment. In case of mould board plowing a violation of soil aggregates takes place, cementation of soil particles depending on precipitation is observed, and the structure stabilizes due to the activity of soil macro-, meso and microfauna (Soane et al., 2012).

Conventional soil treatment with plowing leads to erosion, deflation, dehumification, soil aridization in southern Russian Federation, changing the soil structure and ecosystem related functions of carbon content, greenhouse gas emissions and soil moisture preservation (Valkov et al., 2008; Baybekov, 2018). When reducing the use of conventional tillage, a rational, cost-effective and environmentally friendly technology is needed. These technologies include a number of processing techniques having a minimal impact on the soil of agricultural land by analogy of nature. These nature-like technologies include No-Till. Using No-Till can save on fuel consumption, carbon content and as a result reduce soil erosion processes and increase the carbon – humus content (Tebrügge & Düring, 1999; Soane et al., 2012; Palm et al., 2014; Shekhovtsov & Chaikina, 2018).

This tillage technology is widespread in the United States, Argentina, Brazil and several other countries of the world (Handbook direct sowing, 2004; Soane et al., 2012; Kiryushin, 2013). Due to the absence of mechanical treatment, No-Till preserves the soil structure, soil porosity, and allows retaining water in the soil in the root area, which is necessary for plant growth and development (Uteau et al., 2013). The use of No-Till contributes, along with saving resources, to increasing fertility and reducing the negative impact on agrocenoses. To assess soil treatment techniques for physical condition and structure, it is necessary to take into account the ground relief, amount of precipitation and temperature of the soil (Khitrov & Chechuyeva, 1994; Rusanov et al., 2012; Bogunovic, 2018).

The weak spread of No-Till in Rostov region is due to the conservative thinking of agronomists and the low level of agricultural security with high-performance agricultural equipment, including, among other things, its high cost. According to the ratio of tillage types by 2020 in the structure of the cropped lands of the Rostov region, the largest area of arable land will be processed with surface, shallow processing and No-Till - 43.9%, deep ploughing, chisel and flat-cut treatments – 21.4%, and deep mould board plowing - only 12.5% (Zonal systems, 2013). The use of No-Till technology in the Rostov region has a beneficial effect on the biological state of the soils. When using No-Till, an increase in the carbon content of the post-harvest residues of the plants of the previous harvest is observed; the plant mulch converts the microbiota into humus during its activity (Soane et al., 2012; Zúñiga et al., 2015). No-Till technology has a beneficial effect on the nitrification process of flax, barley and winter wheat (Minnikova et al., 2017a). The nitrification values with the use of the No-Till technology are by 2–3 times higher for flax and barley in comparison to the conventional technology. The maximum nitrification was noted during the period of earing of grain crops and entering the flax into the flowering phase in June. When the soil's agrophysical properties change, not only the nutritional regime of the soil is disturbed, but also the production of enzymes due to the metabolic activity of the soil biota. The activity of soil oxidoreductases and hydrolases in the upper soil layer (0–10 cm) with the use of No-Till depended on the retention of moisture in the soil (Minnikova et al., 2018). Compared to conventional tillage, the closest links are found between hydrothermal indicators and β-fructofuranosidase activity, the activity of which, as it is known, is closely related to soil fertility and humus content (Valkov et al., 2008). Similar results were obtained by

A.V. Zushenitsena et al. (2018) when comparing various technologies for ordinary chernozem treatment in Krasnoyarsk forest-steppe, where increased soil moisture with minimal treatment and No-Till caused a significant increase in cellulose degrading activity by 27–38% and soil respiration by 17–24% (Zushenitsena et al., 2018). The beneficial effect of No-Till technology was confirmed on binary sunflower seeding together with melilot and vetch (Minnikova et al., 2017b). Ordinary chernozem had a high activity of catalase and invertase with all plant crops. In the process of studying binary seeds of sunflower, it was found that the activity of oxidoreductases as a whole decreases with a simultaneous decrease in humidity during the vegetation period, the activity of the protease, on the contrary, increases from June to September.

With different agricultural crops, rainfall and yield were estimated. Crops differ in their methods of sowing and growing with a sowing distance between crops of 2–5 cm (continuous sowing) and 20–25 cm (row crops). In this regard, for agricultural crop, the vegetation period and the amount of rainfall in this period were taken into account. According to the need for critical moisture, row crops, such as sunflower, form a powerful root system, as a result of which they consume moisture from arable (soil layer 0–22 cm) and subsoil soil (deeper than 22 cm), as well as from continuous seeding crops, like winter wheat in arable soil (soil layer 0–22 cm).

The purpose of our study is to assess the effect of productive moisture reserves and rainfall in spring-summer and autumn on agricultural crop yields when using No-Till in the south of the European part of Russian Federation (Rostov region).

MATERIALS AND METHODS

The effect of No-Till on soil quality and field crop yields was investigated in the south of the European part of Russia (Rostov region, Oktyabrskiy district) (Fig. 1). Areas with No-Till and conventional tillage were selected as objects of study.

The soils belong to ordinary chernozems of different thickness (Voronic Chernozems, WRB), degree of leaching from carbonates and humus content (Shishov et al., 2004; Valkov et al., 2008). According to No-Till, chernozems are treated on an area of about 5 thousand hectares over the last 9 years. The territory of the Oktyabrsky district of the Rostov region is located in a region with significant thermal resources. The sum of positive air temperatures (above 10 °C) is 3,200 °C. Summers are hot (temperature July is +24.3 °C),winters are moderately cold (temperature January is -2.5–2.7 °C). At the same time, the major part of the Rostov region belongs to the territory of insufficient and unstable moisture. According to reference data, the average annual rainfall is 423 mm (Khrustalev, 2002).

Figure 1. Location of the research region.

In 2014–2018 years 28 fields with different grown crops were studied: winter wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.), spring barley (*Hordeum* *sativum distichum*), sunflower (*Helianthus annuus* L.), oilseed flax (*Linum usitatissimum* L.), coriander (*Coriandrum sativum* L.), chickpeas (*Cicer arietinum* L.), rape (*Brassica napus* L.). Studies were carried out in grain and grain grass eight-field crop rotations with the following alternation of crops (in % of the area seeding):

1) winter wheat + barley (100%);
2) sunflower + perennial leguminose grasses (80%) + safflower (20%);
3) winter wheat + barley (100%);
4) corn grain (80%) + cruciferous (false flax, rape, mustard) (20%);
5) winter wheat (100%);
6) grain legumes (chickpeas, lentils, peas) (66%) + cruciferous (false flax, rape, mustard) (33%));
7) winter wheat + barley (100%);
8) flax (33%) + coriander (50%) + buckwheat (17%).

Sowing agricultural crops was made with the use of Buhler Versatile Tractor 2375 + Great Plains NTA 3510 (10.7 m) and Case Magnum 315 + Great Plains NTA 3510 (10.7 m). All crops were sown with a row spacing of 19.1 cm. Motor fuel consumption with No-Till amounts to 26 liter per hectare, with CT - 74.1 liter per hectare. When using No-Till in all tillage operations, diesel powered equipment was used meeting international standards for carbon and nitrogen oxide. At the same time, the consumption of diesel fuel for the main tillage at No-Till is 26 liter per hectare, which is 3 times less than with the use of conventional technology (CT). The highest costs were observed at the stage of pre-sowing tillage and harvesting of crops - 31 and 58% respectively, while with the use of CT the main costs at the stage of primary tillage - 44% and harvesting of crops - 20% were observed. No-Till fields are located at a distance of 50–100 m from CT fields.

Sowing agricultural crops was made with the use of Buhler Versatile Tractor 2375 + Great Plains NTA 3510 (10.7 m) and Case Magnum 315 + Great Plains NTA 3510 (10.7 m). All crops were sown with a row spacing of 19.1 cm. With conventional tillage (CT), heavy discs were used harrows of the type BDT-3.0, BDT-7.0, cultivators KPK-8, KPS-4.0 with harrows BZSS-1,0 or BZST-1,0 to a depth of 12–14 cm; the second cultivation – to a depth of 10–12 cm. Motor fuel consumption with No-Till amounts to 26 liter per hectare, with CT - 74.1 liter per hectare. When using No-Till in all tillage operations, diesel powered equipment was used meeting international standards for carbon and nitrogen oxide. At the same time, the consumption of diesel fuel for the main tillage at No-Till is 26 liter per hectare, which is 3 times less than with the use of conventional technology (CT). The highest costs were observed at the stage of pre-sowing tillage and harvesting of crops - 31 and 58% respectively, while with the use of CT the main costs at the stage of primary tillage - 44% and harvesting of crops – 20% were observed. No-Till fields are located at a distance of 50–100 m from CT fields. As the soil surface with direct sowing assessed as weak erosion.

The soil temperature was determined by electronic pocket thermometer HANNA Chectemp at depth of 0–10 cm. Analytical repetition of the analysis execution is 3–10-fold. Soil moisture was determined by volumetric method in the field conditions with humidity meter of Fieldscout TDR 100 in 10-fold repetition in the layer of 0–10 cm at each site. Analytical repetition of the analysis execution is 3–10-fold.

According to the Federal State Statistics Service in 2018, the area under winter wheat and sunflower in the Rostov region in relation to other regions of Russia amounts to 9.3 and 10.3%, respectively. In the Rostov region, winter wheat occupies more than 43–45% of arable land, sunflower - about 15–20%. Winter wheat belongs to the one-year grain-growing agricultural crops of continuous sowing, and sunflower - to the tilled agricultural crops. Agricultural crops differ in the physiological characteristics of the root system structure, the need for moisture, the phases of development, sowing of seeds and agrotechnical manipulations. According to BBCH scale (1991) crops differ in the period of maximum vegetation: in winter wheat these are the phases of stem elongation, heading and full flowering and late milk: from April till June. In sunflower, these phases differ significantly in the growth stage, inflorescence separated, physiological ripeness and over ripe: from June till September. In addition, the ripening of sunflower seeds and winter wheat is influenced by the reserves of productive moisture and the amount of precipitation in the spring-summer and autumn periods.

Yield data for winter wheat and sunflower with No-Till (NT) and conventional technology (CT) are presented by agricultural producers and according to the data of Federal State Statistics Service of Russia. Monthly precipitation for 2014–2018 is presented according to the Rostov-on-Don Weather station.

Statistical processing of the data obtained during the study was carried out using the software package of STATISTICA 12.0. The indicators of variation statistics (mean values, dispersion error of mean), reliability of differences between the variants using dispersion analysis (Student-t) and correlation analysis (Pearson correlation coefficient) were determined.

RESULTS AND DISCUSSION

The total annual precipitation in the Rostov region in 2014, 2015, 2016, 2017 and 2018 was 520, 522, 707, 567 and 722 mm (Fig. 2). In relation to precipitation the years 2014 and 2015 are considered uniformly hydrated during the wheat growing season - 218 and 228 mm in 2014 and 2015 respectively. Substantially less moisture was observed during the growing season of sunflower, which amounted to 65 and 25 mm in 2014 and 2015 respectively.

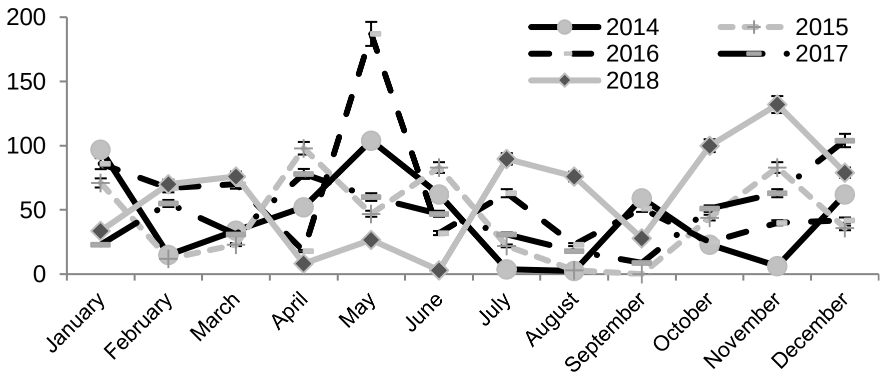


Figure 2. The amount of precipitation in the Oktyabrsky district of the Rostov region in 2014–2018 according to Rostov-on-Don meteorological station.

In 2016 and 2017 the amount of precipitation during the growing season of winter wheat was 237 and 185 mm respectively, and the one of sunflower- 137 and 58 mm. According to the climatic conditions, in 2016 the over precipitation by 66% was shown compared to the normal level, in 2017 – by 34%. In terms of precipitation, 2016 was very wet due to the precipitation in May and July – 187 and 63 mm. In 2016, during the growing season of winter wheat, the precipitation amounted to 237 mm, and the one of sunflower – 169 mm. In 2017, during the growing season of winter wheat the precipitation amounted to 185 mm, the one of sunflower – 105 mm. The greatest amount of precipitation was after the seasons of the critical vegetation of sunflower and winter wheat in October, November and December 2017 – 51, 63, 104 mm, respectively. With an annual high amount of precipitation in the Rostov region (722 mm) in 2018, the growing season of winter wheat was very dry: at a critical growth period from April till June, there was 79–83% less precipitation than in previous years. The lack of precipitation in spring and early summer of 2018 led to a decrease in the yield of winter wheat by 17% throughout Russian Federation, including by 10% in the Rostov region. During the growing season of sunflower (from June till September) there was enough precipitation to form and ripen seeds – 197 mm. Compared with the previous year (2017), there was an increase in the yield of sunflower by 25% in Russian Federation, while only a slight decrease by 10% was observed in the Rostov region.

With the use of No-Till in 2014 and 2015 and the amount of precipitation of 520–522 mm, the yield of winter wheat with No-Till in the Rostov region was higher by 51 and 114% than with the use of CT (Table 1). In 2016–2017 the yield of winter wheat in the Rostov region with the use of No-Till was higher than the one with CT by 36 and 26%, with the overall high yield of winter wheat in the region.

Table 1. Yield of main agricultural crops in the Rostov region with no-tillage (NT) and conventional tillage (CT) (2014–2018), ton·ha^{-1}

Agriculture crop	Type of land use	2014	2015	2016	2017	2018
Winter wheat	NT	36.3	50.7	54.2	56.0	29.8
	CT	24.1	23.7	39.0	44.5	41.0
Sunflower	NT	18.5	27.2	27.5	27.0	25.0
	CT	14.0	14.0	14.3	15.7	19.7

In 2018 due to the drought in the spring-summer period (April-June), the yield of winter wheat decreased by 16% compared with CT and by 46%compared with the harvest of 2017. The prime cost of winter wheat in high-yielding years (2015, 2016 and 2017) was lower than the cost of CT by 41, 45 and 10% respectively. In 2014 and 2015 the yield of sunflower in the study area with the use of NT was higher than the one with CT by 32 and 92%, respectively. Sunflower grown on soils where No-Till was used, in the Rostov region in 2016 and 2017, also had a high yield, especially in 2016 – by 92% compared with CT. Despite the lack of moisture in the growing season of 2014, 2015 and 2017, the yield of sunflower grown with the direct sowing varied in the range of 27.0–27.2 kg ha^{-1}. The yield of sunflower in 2018 compared with 2017 decreased by 10%, but exceeded the yield of the one grown with mouldboard plowing by 27%. Preservation of the harvest field, forming a mulch layer, improving the soil structure and retaining moisture in the soil with technology NT reduces the likelihood of erosion in the soil (Mancinelli et al., 2013; Kazeev et al., 2017; Zhelezova et al., 2017; Mokrikov et al., 2018; Papp et al., 2018). A 3-fold reduction in fuel consumption in case of sowing allows saving motor fuel, which leads to a reduction in greenhouse gas emissions. The reduction of soil treatment stages with NT reduces the soil compaction equipment, which also has a positive effect on the physical and biological state of the soil. Taking into account the decrease in the cost of fuel resources at all stages of sunflower processing, the cost with the use of NT was lower by 35–68% than the one with the conventional technology.

Earlier under the conditions of the production experiment in the agrocenoses of the Oktyabrsky district (Rostov region), on average, for two years, the highest yield was obtained when growing winter wheat by using the NT (4.19 ton ha^{-1}), which is 18% higher than the yield obtained with the use of conventional technology (CT) (Zelensky et al., 2012). The best economic indicators of production were obtained when growing winter wheat with the use of NT, which is due to obtaining the lowest cost of grain and the highest profitability of production. There was also an increase in the yield of sunflower by 49% (22.5 kg ha^{-1}) in the No-Till variant, as compared to mouldboard plowing (Zelensky et al., 2014). This is associated with the increased soil moisture reserves when using NT. In the early spring period, the moisture reserves in the soil layer of 0–150 cm in the NT variant were higher by 15.5 mm, and during the sowing of sunflower - by 20.4 mm. The main indicator of soil restoration when using No-Till includes the accumulation of humus, which is an indicator of fertility due to the activity of the soil microbiota (Kudeyarov, 2015). With a high content of humus and preservation of available moisture in the soil with the use of NT with various agricultural crops, physical properties are improved, the number of soil nitrogen-fixing and cellulose bacteria increases; soil enzymatic activity is stimulated (Soane et al., 2012; Akimenko et al., 2016; Sharkov et al., 2016; Minnikova et al., 2017b; Mokrikov et al., 2017; Kazeev et al., 2017; Shirokikh et al., 2017; Mokrikov et al., 2018).

CONCLUSIONS

NT allows retaining moisture reserves in the soil during the growing season and improves the agrophysical state of the soil, which contributes to an increase in the yield of winter wheat and sunflower. The use of NT can improve the ecological condition of the soil due to the activation of the microbiota, the accumulation of organic matter, the preservation of moisture and the improvement of the structural state. NT results in lower costs due to significant savings in motor fuel. In the long-term dynamics of 2014–2018, when using NT, the cost of winter wheat and sunflower is reduced by 41–45% and 32–92% compared to the CT.

ACKNOWLEDGEMENTS. Supported by the Ministry of High Education and Science of the Russian Federation (Project No 5.5735.2017 / 8.9) and the President of the Russian Federation (Project No NSc-3464.2018.11).

REFERENCES

Akimenko, Yu.V., Mokrikov, G.V., Kazeev, K.Sh. & Kolesnikov, S.I. 2016. *The influence of direct seeding technology on the microbiological properties of chernozem*. Rostov-on-Don: SFU Publishing House, 96 pp.

Bai, Z., Caspari, T., Gonzalez, M.R, Batjes, N.H, Mäderb, P., Bünemann, E.K, Goede, R., Brussaard, L., Xud, M., Ferreirae, S.C.S, Reintam, E., Fang, H., Mihelič, R., Glavan, M. & Tóthi, Z. 2018. Effects of agricultural management practices on soil quality: A review of long-term experiments for Europe and China. Agriculture, Ecosystems and Environment 265.1–7.

Baibekov, R.F. 2018. Nature-like technologies basis for the stable development of agriculture. *Agriculture* **2**, 5–8.
Bogunovic, I., Pereira, P., Kisic, I., Sajko, K. & Sraka, M. 2018. Tillage management impacts on soil compaction, erosion and crop yield in Stagnosols (Croatia). *Catena* **160**, 376–384.
Dridiger, V.K., Kulintsev, V.V., Stukalov, R.S. & Gadzhiumarov, R.G. 2018. Dynamics of changes in the agrophysical properties of the soil in the cultivation of field crops using the no-till technology. *News of the Orenburg State Agrarian University* **5** (73), 35–38.
Gailis, J., Turka, I. & Ausmane, M. 2017. Soil tillage and crop rotation differently affect biodiversity and species assemblage of ground beetles inhabiting winter wheat fields. *Agronomy Research* **15**(1), 94–111.
Handbook of direct sowing on open ground - a special edition of Clarin. Argentina. Artes Gráficas Rioplatense S.A. 2004, 234 pp.
Ivelic-Sáez, J., Zúñiga, F., Valle, S., López, I., Dec, D. & Dörner, J. 2015. Functional resistance and resilience of the pore system of an andisol exposed to different strategies of pasture improvement under sheep grazing. *Soil Science and Plant Nutrition* **15**, 663–679.
Kazeev, K.Sh., Mokrikov, G.V., Akimenko, Yu.V. & Kolesnikov, S.I. 2017. Effect of no-till on the physical properties of south Russia soil. *International Multidisciplinary Scientific GeoConference Surveying Geology and Mining Ecology Management, SGEM* **17**(32). 185–192. doi: 10.5593/sgem2017/32/S13.025
Khitrov, B.N. & Chechueva, O.A. 1994. The influence of plowing and irrigation on the macrostructure of chernozem. *Eurasian soil science* **6**, 106–114.
Khrustalev, Yu.P., Vasilenko, V.N., Svisyuk, I.V., Panov, V.D. & Larionov, Yu.A. 2002. *Climate and agroclimatic resources of the Rostov region*. Rostov-on-Don: Batayskoe Prince. Publishing house, 183 pp.
Kiryushin, V.I. 2013. Problems of minimizing tillage: prospects and objectives of research. *Agriculture* **7**, 3–6.
Kudeyarov, V.N. 2015. The current state of the carbon balance and the limiting ability of soils to absorb carbon in Russia. *Eurasian soil science* **9**, 1049–1060.
Mancinelli, R., Marinari, S., Di Felice, V., Savin, M.C. & Campiglia, E. 2013.Soil property, CO_2 emission and aridity index as agroecological indicators to assess the mineralization of cover crop green manure in a Mediterranean environment. *Ecological Indicator* **34**, 31–40.
Minnikova, T.V, Mokrikov, G.V., Kazeev, K.Sh., Akimenko, Yu.V. & Kolesnikov, S.I. 2017a. The influence of the No-Till technology on the nitrifying activity of the chernozem of the Rostov region. *Agrochemistry* **9**, 33–38.
Minnikova, TV, Mokrikov, G.V., Kazeev, K.Sh., Akimenko, Yu.V. & Kolesnikov, S.I. 2018. Estimation of dependencies between hydrothermal parameters and enzymatic activity of the chernozem of the Rostov region using different agrotechnologies. *Agrophysics* **1**, 9–17.
Minnikova, TV, Mokrikov, G.V., Kazeev, K.Sh., Akimenko, Yu.V. & Kolesnikov, S.I. 2017b. Evaluation of the enzymatic activity of chernozem of the Rostov region under binary sunflower crops. *News of the Timiryazev Agricultural Academy* **6**, 141–155.
Mokrikov, G.V., Kazeev, K. Sh., Akimenko, Yu.V., Myasnikova, M.A. & Kolesnikov, S.I. 2018. Influence of the No-Till technology on the enzymatic activity of different chernozems of Southern Russia. *18th International Multidisciplinary Scientific GeoConference SGEM 2018: Conference Proceedings* **18**, 257–264.
Mokrikov, G.V., Kazeev, K.Sh., Akimenko, Yu.V. & Kolesnikov, S.I. 2017. The influence of direct seeding technology on the ecological and biological properties of black soil. *Izvestiya Vysshikh Uchebnykh Zavedenii. North Caucasus region* **2**, 68–74.
Palm, C., Blanco-Canqui, H., Declerck, F., Gatere, L. & Grace, P. 2014. Conservation agriculture and ecosystem services: an overview. *Agriculture Ecosystems & Environment* **187**, 87–105.

Papp, R., Marinari, S., Moscatelli, M.C., van der Heijden, M.G.A., Wittwer, R., Campiglia, E., Radicetti, E., Mancinelli, R., Fradgley, N., Pearce, B., Bergkvist, G. & Finckhg, M.R. 2018. Short-term changes in soil biochemical properties as affected by subsidiary crop cultivation in four European pedo-climatic zones. *Soil & Tillage Research* **180**, 126–136.

Rusanov, A.M., Milanovsky, E.Yu., Verkhoshentseva, Yu.P., Voropaev, S.B. & Anilova, L.V. 2012. Physical properties of ordinary chernozem in areas of steppe landscapes adjacent to forest areas. *Soil Science* **7**, 763–769.

Sharkov, I.N., Samokhvalova, L.M., Mishina, P.V. 2016. Change in the organic matter of leached chernozem while minimizing processing in the forest-steppe of western Siberia. *Eurasian soil science* **7**, 892–899.

Shekhovtsov, G.A. & Chaykina, N.N. 2018. Soil fertility monitoring. *Agriculture* **6**, 21–26.

Shirokikh, I.G., Kozlova, L.M., Shirokikh, A.A., Popov, F.A. & Tovstik, E.V. 2017. The influence of the method of tillage and biological products on micromycete complexes in the rhizosphere and rhizoplan of spring wheat. *Eurasian soil science* **7**, 837–843.

Shishov, L.L., Tonkonogov, V.D., Lebedeva, I.I. & Gerasimova, M.I. 2004. *Classification and diagnosis of soils of Russia*. Smolensk: Oykumena, 342 pp.

Soane, B.D., Ball, B.C., Arvidsson, J., Basch, G., Moreno, F. & Roger-Estrade, J. 2012. No-till in northern, western and south-western Europe: A review of problems and opportunities for crop production and the environment. *Soil and Tillage Research* **118**, 66–87.

Tebrügge, F. & Düring, R.A. 1999. Reducing tillage intensity – a review of results from a long-term study in Germany. *Soil & Tillage Research* **53**(1), 15–28.

Trofimova, T.A., Korzhov, S.I., Gulevsky, V.A. & Obraztsov, V.N. 2018. Assessment of the degree of physical degradation and the suitability of chernozems to minimize primary tillage. *Eurasian Soil science* **9**, 1125–1131.

Uteau, D., Pagenkemper, S., Peth, S. & Horn, R. 2013. Root and time dependent soil structure formation and its influence on gas transport in the subsoil. *Soil and Tillage Research* **132**, 69–76.

Valkov, V.F., Denisova, T.V., Kazeev, K.Sh., Kolesnikov, S.I. & Kuznetsov, R.V. 2008. *Soil fertility and agricultural plants: environmental aspects*. Rostov-on-Don: SFU publishing house, 416 pp.

Zaushintsena, A.V., Romanov, V.N. & Kozhevnikov, N.V. 2018. The impact of resource-saving methods of primary processing of ordinary chernozem on the indicators of total biological activity and grain yield. *Samara Scientific Bulletin/* 7. **3**(24), pp. 41–48.

Zelensky, N.A., Zelenskaya, G.M., Mokrikov, G.V. & River, Yu.V. 2012. Growing winter wheat using direct sowing technology in the conditions of the Rostov region. *Modern problems of science and education* **6**, 1–5.

Zelensky, N.A., Zelenskaya, G.M., Mokrikov, G.V. & Shurkin, A.Yu. 2018. Soil fertility: the present and the future of our agriculture. A*griculture* **5**, 4–7.

Zelensky, N.A., Zelenskaya, G.M., Shurkin, A.Yu. 2014. Sunflower yield with different technologies of tillage. *Protection and quarantine of plants* **9**, 44–47.

Zhelezova, A.D., Tkhakakhova, A.K., Yaroslavtseva, N.V., Garbuz, S.A., Lazarev, V.I., Kogut, B.M., Kutovaya, O.V. & Kholodov, V.A. 2017. Microbiological indicators of aggregates of typical chernozems in many years of field experiments. *Eurasian soil science* **6**, 711–717.

Zonal farming systems of the Rostov region for 2013–2020 / under total. ed. V.N. Vasilenko. Part 2. S.G. Bondarenko et al. Rostov-on-Don: Ministry of Agriculture and Food of the Rostov Region, 2013, 243 pp.

Zúñiga, F., Ivelic-Sáez, J., López, I., Huygens, D. & Dörner, J. 2015. Temporal dynamics of the physical quality of an Andisol under a grazing system subjected to different pasture improvement strategies. *Soil and Tillage Research* **145**, 233–241.

Agronomy Research **17**(6), 2359–2372, 2019
https://doi.org/10.15159/AR.19.209

Investigation of fly larvae *Lucilia Caesar* application in pet feed composition

L. Nadtochii[1*], D. Baranenko[1], R. Melchakov[1], M. Muradova[1], A. Istomin[2] and A. Istomin[2]

[1]ITMO University, Faculty of Food Biotechnologies and Engineering, Lomonosov street 9, RU191002 Saint-Petersburg, Russia
[2]Zooprotein LLC, Soviet street 64, RU398042 Lipetsk, Russia
[*]Correspondence: l_tochka@itmo.ru

Abstract. The biomass of insect larvae is world-widely used as a valuable raw material for the pharmaceutical, microbiological, cosmetic industry and feeding production, also in the food industry. There is certain complex technology for processing biomass of insect larvae, which affords to isolate many physiologically active substances - chitin, antimicrobial peptides, fatty acids mixture, organic forms of mineral substances, hormones, etc.
The company New Biotechnology (Lipetsk, Russia) has developed a technical process for producing of the protein-lipid preparation (commercial name is Zooprotein) based on the fly larvae of the species *Lucilia Caesar*. The utilization of food waste as a substrate, unpretentiousness to cultivating environment and high protein content are capable of considering insects of the species *Lucilia Caesar* as a promising object of cultivation and a reliable, cheap, replenishable source of nutrients for resource-saving process of the feed production.
On the bases of ITMO University, an investigation is being conducted on the qualitative composition of the Zooprotein and the possibility of pet feed application. Cats are the most demanding animals to the quantitative and qualitative composition of protein fractions of feed. In present research an evidence-based calculation of the balance of the Zooprotein composition is presented as a feed component for cats during growth. Accordingly, the unique chemical composition of the development product based on fly larvae *Lucilia Caesar* makes possible to maintain that it is a promising functional ingredient in feeding rations for various animal species.

Key words: fly larvae, *Lucilia Caesar*, feed production, protein sources, raw materials; nutrients, fatty acids, amino acids, protein-lipid preparation, energy, pets.

INTRODUCTION

At the present time, the production of prepared animal feed is one of the leading sectors of the agro-industrial complex that includes manufacturing of animal feed, feed additives, drugs, etc., and is based on modern achievements of microbiological, pharmaceutical, food, chemical and other manufacturing sectors (Afanasyev, 2012).

About 4 billion tons of animal feed is produced annually in the world, whereof only 600 million tons are creep feed according to the International Federation of the Feed Industry (FAO, 2018).

Nowadays, secondary raw ingredients of animal and vegetable origin are widely used in feed production: whey, buttermilk, meat, bone, blood and fish meals, vegetable oil cake, etc. (Sencic et al., 2011). The main components of plant origin, which are contain high level of carbohydrates for animal feed are: grain varieties (wheat, barley, oats, millet, triticale, corn) – up to 85%; oilseed meal (flax, soybean, sunflower) – up to 15–25%; legumes with a high protein content (soybeans, beans, peas, chickpeas, lupins) – up to 45%; oilseeds (rapeseed, sunflower, cotton, camelina, colza) – up to 15%; hay, straw, other high-fiber roughage; grain and food industry waste; amino acids; mineral mixtures; vitamin supplements; antibiotics and biostimulants (Yasir et al., 2017). However, the use of secondary food raw materials for the production of feed is currently in competition with their involvement in the composition of food products. The use of biotechnology makes it possible to obtain valuable food ingredients and food products from secondary raw materials of animal and vegetable origin (Kuznetsova et al., 2014; Suchkova et al., 2014). Thus, the use of secondary food resources for the production of products with higher added value reduces the attractiveness of this raw material for feed production.

Increased world population, environmental effects, augmented land use resources and growth of demand for nutrients and non-renewable energy are predicted for coming decades. About 70% of all agricultural land uses is livestock production (Zanten et al., 2016) The global demand for livestock products is expected to almost double by 2050, thus innovative production solutions are required. Insect farming has been suggested as a good alternative to conventional livestock farming for future feed production (Van, 2013).

Growing insects in special farms under controlled conditions has several advantages. One of the advantages is the possibility of growing insects based on organic waste, which confirms the environmental and resource-saving aspects of this production. In addition, insects farming produces less greenhouse gases and ammonia, for instance, compared to cattle or pigs (Halloran et al., 2014). Cultivation of insects is also economically advantageous, since this type of production requires little resources in terms of land and water as contrasted with the cost of breeding cattle, etc.

However, at present, the cultivation of insects has not received a wide scale in the world. Most often, industrial enterprises of insect farming are a family business, and their main focuses are growing flour worms, crickets and grasshoppers for feeding animals in zoos and processing them for pet food (Belluco et al., 2013).

Aquaculture (fish farming, crustaceans and other aquatic animals) is one of the fastest extending industries. Nevertheless, the main obstacle to sustainable growth in the industry is the feed cost, in particular fish meal and fish oil (Van, 2013). Approximately 10% of fish products are processed into fish meal, and stocks of ocean fish are depleted due to overfishing as feed. The need for effective competition in this aspect indicates the feasibility of finding new sources of raw materials for feed production, for instance, various types of insects (Raubenheimer & Rothman, 2011).

The urgency of finding alternative feed of animal origin in regard to fish meal and soy meal has led to the recognition of insect protein in the market (Van et al., 2015). Insect-based feed products can provide an alternative to fish meal and soy meal, which are the most popular components used in feed mixtures for aquaculture and animal husbandry (Nadtochii et al., 2017) That fact creates prerequisites for investigating the possibility of using insects, which are widely distributed in nature, as a renewable source of protein in reference to feed industry, that has become more cost-effective. Studies on livestock have shown that insect meal is poised to replace fish meal in feed, which reduce overfishing (Makkar et al., 2014).

One of the advantages of using insects as an alternative source of protein in feed is that some insects recycle waste material into an extremely useful animal protein.

Many researchers have reported the insect larvae application as a renewable source of protein for pigs, birds, and fish feeding (Awoniyi et al., 2004; Charlton et al., 2015; Schiavone et al., 2017).

It is estimated that there are about 2,000 species of edible insects in the world; of these species, those that are considered most suitable for animal feed production include worms (*Tenenbrio Molitor*, *Zophobas Morio*, *Alphitobus Diaperinus),* and the larvae of the black soldier fly (*Hermetia Illucens*) or house flies (*Musca Domestica*) (Jongema, 2014).

Insects are a source of energy, protein, fat, minerals and vitamins, while the energy content is on par with other sources of fresh meat (based on fresh weight); the exception is pork due to its high fat content (Rumpold & Schluter, 2013a). The average estimates show that energy levels are around 400–500 kcal per 100 g of dry matter, which makes it comparable to other protein sources (Rumpold & Schluter, 2015). It has been reported that the inclusion of insects in broiler chicken feed will not reduce growth rates, and in some cases increase the growth rates of chickens (Rumpold & Schluter, 2013b). The best feed conversion was observed in chickens fed insects (Marono, 2017).

Many scientists have concluded in their research that fly larvae meal has a suitable nutritional composition and can serve as a substitute for fish meal, as well as other sources of protein commonly used in animal nutrition (Ramos et al., 2002; Magalhaes et al., 2017; Wang et al., 2017).

Table 1 is represented a comparison between fish meal, sunflower meal, soy meal and fly larvae meal. As can be seen from this table, the fly larvae meal surpasses some other traditional protein sources used in animal feeding, but, in some cases, is inferior (Aniebo et al., 2011; De Koning, 2005).

Table 1. Comparison of the nutritional value of fly larvae meal with indicators of other commonly used protein sources

Indicators	Fly larvae meal	Fish meal	Soy meal	Sunflower meal (after squeezing
Composition (dry matter), %				
Crude protein	50.86	68.84	49.44	35.56
Essential extract	27.32	5.66	0.45	1.22
Crude Fiber	8.10	1.07	7.87	26.67
Ash	6.75	20.38	7.64	–
Amino acid profile:				
Lysine	6.52	8.86	3.02	1.11
Histidine	3.34	2.88	1.31	0.61
Threonine	2.19	5.34	1.93	1.17
Arginine	6.26	7.04	3.53	2.56
Valin	3.90	6.83	2.33	1.78
Methionine	2.46	2.35	0.70	0.56
Isoleucine	3.30	5.55	2.20	1.11
Leucine	6.86	8.00	3.81	1.78
Phenylalanine	4.28	4.91	2.43	1.28
Tryptophan	–	1.07	0.83	0.50
Cystine	0.56	4.48	0.74	0.56
Tyrosine	3.14	4.70	2.15	–

In Table 1 is shown that the fly larvae meal has a high content of crude protein than soy and sunflower meal, but lower than fish meal. The fly larvae meal is represented by an excellent amino acid composition as well. Besides the fly larvae meal has higher concentrations of histidine and methionine compounds than fish meal.

Insects are able to thrive on a wide range of substrates, for instance, food waste consisting of animal and vegetable waste (Surendra et al., 2016). These food wastes can be used in order to breed insects, that are a cheap and even income-generating substrate. In addition, studies have shown that one of the insect species allowed for use as animal feed, larvae of black soldier flies (*Hermetia Illucens*) reduced organic waste by 60% in 10 days (Salomone et al., 2017). The fly larvae can be grown on a wide range of wastes thereby offering a solution to the growing problem of organic waste. In concordance with above mentioned, protein production for livestock feed may be derived from by-products and waste resulting from human consumption (Diener et al., 2009).

There are several key ways that agricultural insects could be a profitable alternative for agricultural protein production. Mass insect cultivation requires 50–90% less land than conventional agriculture per kilogram of protein, and can reduce greenhouse gas emissions from livestock about 50% by 2050 (Tilman & Clark, 2014).

The fly larvae of the species *Lucilia Caesar* are unpretentious to the conditions of cultivation, in particular, they are grown on food waste as a substrate. Despite this, the results of a previous study were obtained, which showed the antimicrobial activity of *L. caesar* larvae using indicator microorganisms, which are pathogenic for humans and birds (*Salmonella enteritidis, Staphylococcus aureus, Enterococcus faecium, Listeria monocytogenes, Clostridium perfringens, Escherichia coli 015, Pseudomonas aeruginosa*). Russian scientists have shown that growing *L. caesar* fly larvae on forcemeat, artificially contaminated, caused growth inhibition of pathogenic bacteria from the substrate. The obtained results on the antimicrobial activity of *L. caesar* larvae towards a number of bacterial infections in humans and birds allows us to consider the protein-lipid preparation from dried and crushed fly larvae of the species *Lucilia Caesar* as a promising feed production resource for pets admissible to the safety standards (Teymurazov et al., 2018).

However, the use of organic waste as a feed ingredient for insects is another legislative issue. In the short term, legislation in frame of European Union (EU) does not allow for the use of protein from insects as feed for animals. The Transmissible Spongiform Encephalopathies regulation at the European level also blocks the introduction of insects as an ingredient for feed (Regulation (EC) No 999/2001). Currently, in other regions the use of fly larvae protein is an acceptable procedure where this practice has long been conducted and large-scale production has been established. The largest producers of fly larvae protein as an additive in pet feed are regions of North and South America, Eastern Europe, Asia Pacific, Middle East and Africa (Kone et al., 2017; Kenis et al., 2018).

In this regard, the search for new forms of high-quality and safe raw materials are the overriding priority of productive feed production.

In present study, we consider the possibility of using the larvae of the *Lucilia caesar* flies as a raw ingredient in the production of high-quality feed for adult cats.

MATERIALS AND METHODS

Sampling

As objects of research was used following raw ingredients:

– fish meal in accordance with GOST (the State Standard of the Russian Federation) No 2116/2000 'Feed meal from fish, marine mammals, crustaceans and invertebrates'.

– meat and bone meal in accordance with GOST (the State Standard of the Russian Federation) No 17536/1982 'Feed meal of animal origin'.

– the protein-lipid preparation (commercial name – 'Zooprotein') was manufactured at the enterprise 'New Biotechnologies' LLC (Lipetsk) according to the technical specifications. This company specializes in the production of high-protein product from dried and crushed fly larvae of the species *Lucilia Caesar*.

The fly larvae cultivation

Growing fly larvae (species *Lucilia Caesar*) was carried out in a special chamber (cage) by placing eggs from adult flies on a nutrient medium. The nutrient medium served as crushed meat obtained from a forced killed and fallen bird, crushed meat was supplied by the poultry factory of the Lipetsk region (Russia). The nutrient medium was used for growing the fly larvae in the amount of a daily portion 1,000 ± 5% grams per control group (Teymurazov et al., 2018).

The fly larvae incubation was implemented without special lighting at 25 ± 2 °C with 70% relative humidity for 5 days. Throughout the process, constant air ventilation was maintained to prevent the accumulation of heat, to provide a certain level of oxygen and to remove excess CO^2. According to data (Liland et al., 2017), the larvae amount per cage was maintained at an average 830,000 larvae m^{-3}.

The manufacture of the protein-lipid preparation 'Zooprotein'

The fly larvae samples were subjected by heat treatment at 60–70 °C during the day until the mass fraction of moisture was no more than 4%. As a result, dried samples of the preparation Zooprotein were obtained, which were packed in sterile plastic containers and transported to the laboratory for further investigation.

Determination of amino acid composition of the protein-lipid preparation 'Zooprotein'

The tests were carried out at the laboratory of the organization Limited Liability Company Research and Testing Center 'CHERKIZOVO' on the amino acid analyzer HITACHI L–8900.

The total content determination of (free and bound forms) of individual amino acids was conducted according to GOST (the State Standard of the Russian Federation) No 32195/2013 'Feed, all-mash. Method for determination of amino acid content'.

Determination of fatty acid composition of the protein-lipid preparation 'Zooprotein'

Tests to determine the fatty acid composition of the preparation Zooprotein were carried out at the laboratory of the organization Limited Liability Company Research and Testing Center 'CHERKIZOVO' (Russia), according to the procedure described below.

A drop of fat (5 mg) was diluted with 1 ml of dichloromethane, 40 μl of BSTFA was added, kept for 4 hours and analyzed on a chromatography-mass spectrometry system including a Finnigan Trace GC Ultra gas chromatograph and a Finnigan PolarisQ mass spectrometer. The analysis was performed in the mode without dividing the flow with the start of purging after 0.1 min; the temperature of the injector (sampling device) was 260 °C; helium carrier gas flow rate was 1 mL min^{-1}. Column is DB–5 ms 30 m–0.25 mm–0.25 μm. The initial temperature of the chromatographic oven was 60 °C (2 min), then sample was heated to 310 °C at a rate of 6 °C min^{-1} and kept at this temperature for 12 min. The interface and the ion source temperatures of the mass spectrometer were 230 °C and 220 °C, respectively. The mass range is 40–550 atomic mass units (amu). Identification was performed by expert analysis using the NIST MS mass spectra collection. The assessment of the relative content was carried out according to the method of internal normalization.

Calculating BMR:

Basal metabolic rate (BMR) is the daily energy amount expended in the period of sleep in 12–18 hours after eating under normal temperature conditions. For cats and dogs weighing more than 2 kg, the amount of basal energy is determined by the following formula:

$$\text{BMR (kcal day}^{-1}) = 30 \times \text{body weight}^{*} + 70 \tag{1}$$

where *the body weight of an animal is 2.5 kg.

Calculating MER:

Maintenance energy requirements (MER) are determined by the amount of energy consumed by a moderately active animal under thermoneutral conditions. Maintenance energy for cats weighing more than 2 kg (kcal day^{-1}) is following:

$$\text{MER} = 1.4 \times \text{BMR} \tag{2}$$

Calculating ME:

The daily requirement for the animal's metabolizable energy (kcal day^{-1}) is calculated considering the increase ratio in the energy needs of the animal. The increase ratio in the energy needs of the cat during the growth period (3–6 months) is 1.6. Thus, the daily needs for metabolizable energy of a cat (3–6 months of age) is equal to:

$$\text{ME} = 1.6 \times \text{MER} \tag{3}$$

Calculating metabolizable energy (ME in feed):

For predictive equations of ME in prepared pet foods for cats and dogs (dry and wet) the following 4-step-calculation can be used (NRC 2006):

$$\begin{gathered}\text{Gross Energy (GE), kcal} = (5.7 \times \%\ \text{protein}) + (9.4 \times \%\ \text{fat}) + \\ +[4.1 \times (\%\ \text{NFE}^{**} + \%\ \text{crude fibre})]\end{gathered} \tag{4}$$

$$\text{Energy Digestibility (ED), \%} = 87.9 - (0.88 \times \%\ \text{crude fibre in DM}) \tag{5}$$

$$\text{Digestible Energy (DE), kcal} = (\text{GE} \times \text{ED}) \div 100 \tag{6}$$

$$\text{Metabolizable Energy (ME in feed), kcal} = \text{DE} - (0.77 \times \%\ \text{protein}) \tag{7}$$

where **NFE – Nitrogen free extract is 0.4.

The satisfaction degree assessment of the daily need for macro- and micronutrients

$$\text{Units 1,000 kcal}^{-1} = \frac{\text{Nutrient requirement per day (Units kg}^{-1}\text{metabolic BW)} \times 1{,}000}{\text{DER (kcal kg}^{-1}\text{metabolic BW)}} \quad (8)$$

Statistical analysis

Studies of the chemical properties of the samples were carried out in triplicate, the data were processed by the method of mathematical statistics with using MS Excel, finding the confidence interval and a probability of 0.95.

RESULTS AND DISCUSSION

Production waste can potentially be used to grow insects, in particular fly larvae (Diener et al., 2009). A comprehensive scheme for waste processing industry using fly larvae (species *Lucilia Caesar*) is represented in Fig. 1, which makes it possible to turn a large amount of organic waste into fertilizers for the agricultural industry, as well as protein-rich biomass, which is considered as an alternative raw material source for feed production. A comparative analysis of the chemical composition of traditional raw materials for feed production is represented in Table 2.

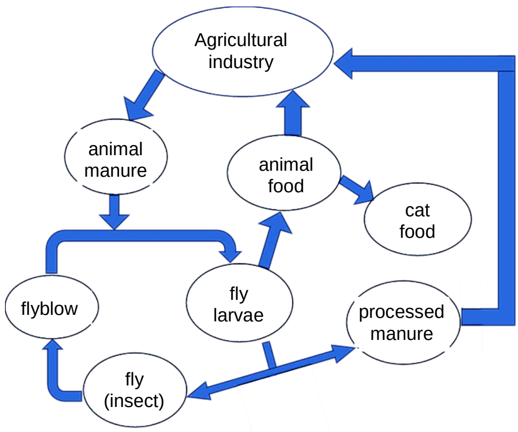


Figure 1. Resource-saving technologies based on fly larvae.

Based on the data in Table 2, it can be noted that Zooprotein is slightly inferior to fish meal and meat and bone meal in terms of crude protein (by 5.8%) and fat (by 2.4 and 1.4%, respectively), however is significantly superior in fiber content (by 8.1 and 6.1%).

According to the analysis of the table data, Zooprotein can be considered as a raw ingredient in animal feed. In present research, this hypothesis was evaluated from thepoint of view to satisfy the needs of cats, taking into consideration that cats are carnivorous and most demanding on the protein-lipid composition of feeding rations. Specific differences in the cat nutrition

Table 2. The chemical composition of raw materials for feed production

Indicators	Raw material resources, g 100 g^{-1} sample		
	Fish meal	Meat and bone meal	Zooprotein
Crude protein, no less than	50.0	50.0	44.2
Crude fat, no more than	14.0	13.0	11.6
Crude fiber, no more than	0.0	2.0	8.1
Moisture content, no more than	12.0	12.0	12.0

compared with dogs are due to behavioral, anatomical, physiological and metabolic features (Hill et al., 2009). The diet of cats is distinguished by an increased need for protein as contrasted with the diets of dogs, in particular, cats require 50% more protein during their growth period and in adult status.

The studied samples are considered as dry feed for kittens during their intensive growth (at the age of 6 months), when there is an increased need for proteins, fats, etc. Based on the available data, the daily need for six months old kittens at body weight 2.5 kg is 100 g for female animal and 110 g for male animal of dry feed per day (Lewis et al., 1987). Table 3 is shown the calculation of animal's need satisfaction in the nutrients calculated as 100 g of dry matter due to the consumption of the test samples during the growth period.

Table 3. The cat's need satisfaction in nutrients, per day

Estimated factor	Cat needs for growth, pregnancy and lactation day^{-1}	Fish meal	Meat and bone meal	Zooprotein
Gross Energy, kcal g^{-1} of dry matter	≥ 4.5	4.7*	4.7*	4.5*
Protein, g 100 g^{-1} of dry matter	> 35.0	56.8*	56.8*	50.2*
Fat, g 100 g^{-1} of dry matter	≥ 17.0	15.9	14.8	13.2
Fiber, g 100 g^{-1} of dry matter	< 5.0	0.0	2.3*	9.2**

*completely satisfies the need in nutrient; **exceeds nutrient requirements.

The daily requirement for the animal's metabolizable energy (ME) was determined to 325 kcal day^{-1}, which is based on the calculation of the basal metabolic rate (BMR), equal to 145 kcal day^{-1}, and maintenance energy requirements (MER), equal to 203 kcal day^{-1}.

The metabolizable energy (ME in feed) was calculated separately for each test sample: fish meal – 372.29 kcal, meat and bone meal – 363.36 kcal and Zooprotein – 316.5 kcal per 100 g sample. The metabolizable energy (ME in feed) based on calculation of following indicators: gross energy (GE), equal for fish meal – 474.9, for meat and bone meal – 472.7, for Zooprotein – 445.1 kcal per 100 g; energy digestibility (ED), equal for fish meal – 87.9, for meat and bone meal – 85.9, for Zooprotein – 79.8; digestible energy (DE), equal for fish meal – 416.03, for meat and bone meal – 407.1, for Zooprotein – 355.2 kcal. Consequently, the daily requirement in feed for providing the animal with the necessary energy amounted to 87.3 for fish meal, for meat and bone meal – 89.4, for Zooprotein – 102.7, which corresponds to the need in feed for kittens aged 6 months, equal to 100–105 g.

Thus, Zooprotein is the balanced feed resource for cats and not inferior to other studied samples. However, it is important that the animal receives a balanced amount of energy and nutrients with the feed including essential feed components. If animals receive a diet that is unbalanced there may be a shortage of all nutrients. This deficiency is especially often observed during the period of growth and during lactation, in the case of feeding animals with low-calorie industrial feed or homemade ration. In addition, to satisfy the energy needs, the animal is forced to eat a large amount of feed. As a result, the kitten's belly hangs down, growth slows down, etc. (Baker, 1991).

Since cats are carnivores, it is especially significant to study their needs for amino acids by the consumption of various types of raw materials. It is important to evaluate the cats needs for certain amino acids, in particular for arginine, during their growth. In this regard, the usual diet of cats should contain many animal components with a fairly high protein content, including arginine. Table 4 is represented the protein component of Zooprotein, consisting of 10 amino acids, among which the arginine, methionine and tryptophan are indispensable in feeding kittens.

Table 4. Amino acid composition of Zooprotein

Amino Acids	The content of essential amino acids in Zooprotein	
	g 100 g^{-1} of protein	g 100 g^{-1} of product
Arginine	3.03	1.34
Histidine	1.97	0.87
Isoleucine	3.51	1.55
Leucine	5.63	2.49
Lysine	6.04	2.67
Methionine	1.92	0.85
Methionine + cystine	2.83	1.25
Phenylalanine	5.77	2.55
Threonine	3.55	1.57
Tryptophan	1.11	0.49
Valine	4.73	2.09

The lipid component of the feed. is not a primary factor in feeding cats However, investigations have been clearly shown that domestic cats cannot synthesize linoleic acid (LA) and have limited ability to synthesize arachidonic acid (AA) during metabolism (Bukkens et al., 1997; Bauer, 2004).

The most important essential fatty acid is linoleic acid (omega-6). It was found that it is an important dietary component necessary for the growth and prevention of skin lesions in animals. Linoleic acid (omega-6) can be converted into long chain polyunsaturated fatty acids that have additional functions as precursors of eicosanoids,which are powerful physiological mediators of cellular functions. Thus, essential fatty acids in the diet of cats are naturally polyunsaturated fatty acids. This group includes linoleic acid (LA), α-linolenic acid (ALA) and, under certain conditions, docosahexaenoic acid (DHA) and arachidonic acid (AA).

The Table 5 is shown the results of the lipid component of Zooprotein, which is confirmed by the data in Fig. 2. The research results confirm that Zooprotein is a source of necessary linoleic acid (omega-6) in the diets of feeding kittens, which is contained in the amount of 0.48 g per 100 g of Zooprotein.

Table 5. Fatty acid composition of Zooprotein

Lipid compounds	Content ratio, %
n-paraffins	2.20
Fatty acids:	
C3:0	0.88
H-C12:0	0.05
C14:1	0.03
H-C14:0	0.43
H-C15:0	0.06
C16:2	0.09
C16:1	4.04
н-C16:0	7.52
н-C18:0	4.57
C18:1	20.71
C18:2	0.48
C22:1	2.8
Other compounds	
Trimethylphosphate	1.70
Cholesterol	7.64
Cholest-5-en-3-ol	3.98
Cholesterol derivative	0.78
3a-Methoxy-9b-lanosta-7.24-dien-26.23-olide	1.74

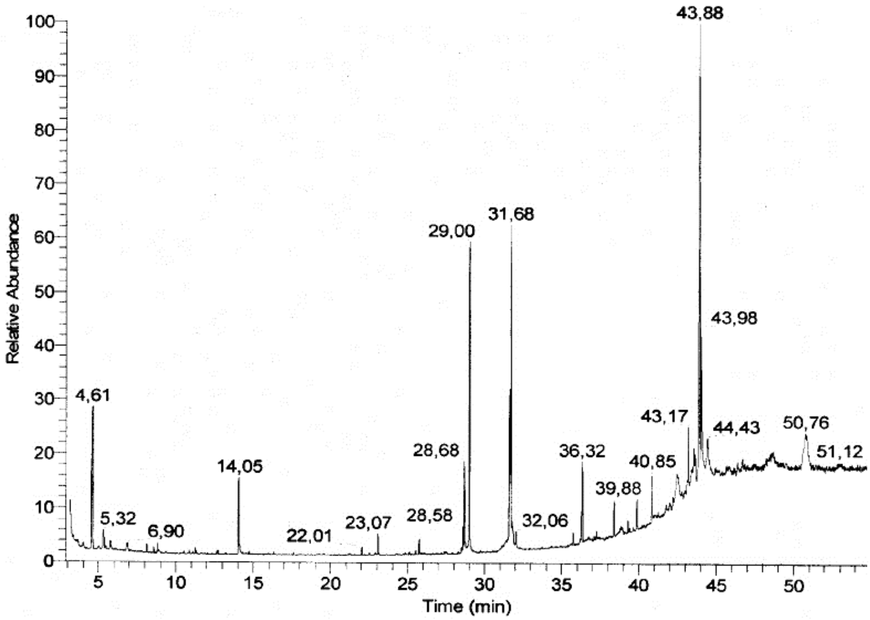


Figure 2. Mass chromatogram of Zooprotein lipid compounds on the full ionic current.

In present research the reference data were used for assessing the need satisfaction of kittens in nutrients during their growth period (FEDIAF, 2017). In Table 6 is represented data on the needs of kittens in the nutrients in terms of daily metabolizable energy, which was examined earlier and was equal to 325 kcal. The daily portion of Zooprotein is 102.7 g which provides the kitten's need for energy.

The nutrient levels for cats during growth are noted in a separate column: minimum recommended nutrient levels (on the left) and maximum recommended nutrient levels (on the right and indicated by *) and legal maximum nutrient levels for sodium (on the right and indicated by **). Legal maximum in EU legislation is expressed on 12% moisture content and there is not account for energy density.

Based on the data in Table 6, we can conclude that Zooprotein is an excellent source of amino acids, fatty acids and minerals. In particular, the daily portion of Zooprotein satisfies the minimum requirement for kittens in 11 amino acids by more than 100%. In addition, the daily dose of Zooprotein partially satisfies the animal's need for the maximum recommended amount of certain essential amino acids, in particular 48.59% arginine, 83.02% methionine and 36.96% tryptophan. The kitten's need of for essential linoleic fatty acid (ω-6) is satisfied by 111.11%, taking into account the daily intake of Zooprotein. The minimum need in minerals is satisfied over 100% at the level of potassium (138.78%) and sodium (353.85%) and partially at the level of calcium (65.43%) and phosphorus (88.24%), and the sodium levels in the daily portion of Zooprotein provide 37.71% of the kitten's need for this mineral.

It is obvious that Zooprotein outperforms other studied samples in a number of indicators (energy value, proteins (amino acids), fats (essential fat acid), fibers and minerals.

Table 6. The nutrient levels for need satisfaction of kittens with consideration to the daily portion of Zooprotein

Major nutrients	Minimum recommended nutrient levels for cats during growth (except * and **)		The content of nutrients in 102.7 g of Zooprotein (daily portion)	The level of satisfaction of the nutrients need, %
	g 1,000 kcal^{-1} of ME	g 325 kcal^{-1} of ME		
Amino Acids (Essential Amino Acids)				
Arginine	2.78 **(8.75*)**	0.90 **(2.84*)**	1.38	153.33 (**48.59**)
Histidine	0.83	0.27	0.89	329.63
Isoleucine	1.35	0.44	1.59	361.36
Leucine	3.2	1.04	2.56	246.15
Lysine	2.13	0.69	2.74	397.10
Methionine	1.10 **(3.25*)**	0.36 (**1.06***)	0.88	244.44 (**83.02**)
Methionine + cystine	2.20	0.72	1.29	179.17
Phenylalanine	1.25	0.41	2.62	639.02
Threonine	1.63	0.53	1.62	305.66
Tryptophan	0.40 **(4.25*)**	0.13 (**1.38***)	0.51	392.31 (**36.96**)
Valine	1.6	0.52	2.15	413.46
Taurine (dry pet food)	0.25	0.08	0.24	300.00
Essential Fat Acid				
Linoleic acid (ω-6)	1.38	0.45	0.50	**111.11**
Minerals				
Calcium	2.50	0.81	0.53	65.43
Phosphorus	2.10	0.68	0.60	88.24
Potassium	1.50	0.49	0.68	138.78
Sodium	0.40 **(3.75**)**	0.13 **(1.22**)**	0.46	353.85 **(37.71)**

* N – Recommended maximum, not harmful to the animal; ** – Scientific data show that sodium levels up to 3.75 g per 1,000, kcal ME are safe for healthy cats. Higher levels may still be safe, but no scientific data are available.

A comparative analysis of the prime cost of the studied samples was examined, in particular, the indicator for fish meal was 1,320–1,590 euro ton^{-1}, for meat and bone meal – 0,530–0,660 euro ton^{-1}, Zooprotein – 1,320 euro ton^{-1}. Thus, the competitive cost of Zooprotein proves the perspectivity of its use in combination with traditional feed raw materials, which can be widely used in food production.

CONCLUSIONS

This research confirms that Zooprotein is a high-quality feed resource in countries where using of fly larvae protein is an acceptable procedure. It has been established that the energy requirements for kittens at the age of 6 months, it is enough for them to consume 102.7 g of Zooprotein per day. The consuming of the daily portion of Zooprotein allows to satisfy the needs of kittens in most essential nutrients, such as essential amino acids (arginine, methionine, tryptophan, taurine etc.), essential fat acid (linoleic acid) and minerals (calcium, phosphorus, potassium, sodium). The competitive prime cost of Zooprotein also allows us to consider it as a promising raw material resource. In future research, it is advisable to find an optimal recipe for cat feed based on several raw ingredients using the method of multivariate modeling.

ACKNOWLEDGEMENTS. This work was financially supported by the government of the Russian Federation, Grant RFMEFI58117X0020.

REFERENCES

Afanasyev, V.A. 2012. The current state and development prospects of the feed mill industry of the Russian Federation. *All-Russian Research Institute of the feed mill industry* **3**, 116–124 (in Russian).

Aniebo, A., Odukwe, C.A., Ebenebe, C.I., Ajuogu, K., Owen, O.J. & Onu, P.N. 2011. Effect of housefly larvae (*Musca domestica*) Meal on the carcass and sensory qualities of the mud catfish, (*Clarias gariepinus*). *Adv. Food Energy Secur*. **1**, 24–28.

Awoniyi, T.A., Adetuyi, F.C. & Akinyosoye, F.A. 2004. Microbiological investigation of maggot meal, stored for use as livestock feed component. *Journal of Food, Agriculture & Environment* **2**, 104–106.

Baker, D.H. 1991. Comparative nutrition of cats and dogs. *Annu Rev Nutr*. **11**, 239–63.

Bauer, J.E. 2004. Lipoprotein-mediated transport of dietary and synthesized lipids and lipid abnormalities of dogs and cats. *American Veterinary Medical Association* **5**, 668–675.

Belluco, S., Losasso, C. & Maggioletti, M. 2013. Edible insects in a food safety and nutritional perspective: a critical review. *Comprehensive Reviews in Food Science and Food Safety* **12**, 296–313.

Bukkens, S.G.F. 1997. The nutritional value of edible insects. *Ecol. Food Nutr*. **36**, 287–319.

Charlton, A.J., Dickinson, M., Wakefield, M.E., Fitches, E., Kenis, M., Han, R., Zhu, F., Kone, N., Grant, M., Devic, E., Bruggeman, G., Prior, R., Smith, R. 2015. Exploring the chemical safety of fly larvae as a source of protein for animal feed. *Journal of Insects as Food and Feed* **1**, 7–16.

De Koning, AJ. 2005. Properties of South African fish meal. A review. *South African Journal of Science* 101 pp.

Diener, S., Zurbrugg, C. & Tockner, K. 2009. Conversion of organic material by black soldier fly larvae: establishing optimal feeding rates. *Waste Manag. Res*. **6**, 603–610.

FAO. 2018. Food and agriculture organization of the United Nations statistical databases. Available at: http://www.fao.org/statistics/en/

GOST (the State Standard of the Russian Federation) No 17536/82. Feed meal of animal origin. 1982. *Technical specifications*, pp.1–5. (in Russian).

GOST (the State Standard of the Russian Federation) No 2116/2000. Feed meal from fish, marine mammals, crustaceans and invertebrates. 2000. *Technical specifications.* 1–14 (in Russian).

GOST (the State Standard of the Russian Federation) No 32195/2013. Feed, all-mash. Method for determination of amino acid content. 2013. *Interstate Council for Standardization, Metrology and Certification*, pp. 1–23 (in Russian).

Halloran, A., Muenke, C., Vantomme, P. & Huis, A.V. 2014. Insects in the human food chain: global status and opportunities. *Food Chain*. **2**, 103–118.

Hill, R.C., Choate, C.J., Scott, K.C. & Molenberghs, G. 2009. Comparison of the guaranteed analysis with the measured nutrient composition of commercial pet foods. *J Am Vet Med Assoc*. **234**, 347–51.

Jongema, Y. 2014. List of edible insect species of the world. Wageningen: Laboratory of Entomology, Wageningen University, The Netherlands. Available at: http://www.entwurnl/UK/Edible+insects/Worldwide+species+list/

Kenis, M., Bouwassi. B., Boafo, H., Devic, E., Han, R., Koko, G., Koné, N., Maciel, G., Nacambo, S., Pomalègni, S.C.B., Roffeis, M., Wakefield, M., Zhu, F. & Fitches, E. 2018. Small-Scale Fly Larvae Production for Animal Feed. *Edible insects in Sustainable Food Systems*, pp. 239–261.

Kone, N., Sylla, M., Nacambo, S. & Kenis, M. 2017. Production of house fly larvae for animal feed through natural oviposition. *Journal of Insects as Food and Feed* **3**, 1–10.

Kuznetsova, L., Zabodalova, L. & Baranenko, D. 2014. On the potential of lupin protein concentrate made by enzymatic hydrolysis of carbohydrates in dairy-like applications. *Agronomy Research* **12**(3), 727–736.

Lewis, L.D., Morris, M.L. & Hand, M.S. 1987. *Small animal clinical nutrition*. Mark Morris Associates Topeka, Kansas, 144 pp. (in Russian).

Liland, N.S., Biancarosa, I., Araujo, P., Biemans, D., Bruckner, C.G., Waagbo, R., Torstensen, B.E. & Lock, E.J. 2017. Modulation of nutrient composition of black soldier fly (Hermetia illucens) larvae by feeding seaweed-enriched media. *PLoS One* **12**(8), 24 pp.

Magalhaes, R., Sanchez-Lopez, A. & Leal, R. 2017. Black soldier fly (*Hermetia illucens*) pre-pupae meal as a fish meal replacement in diets for European seabass (Dicentrarchus labrax). *Aquaculture* **476**, 79–85.

Makkar, H.P.S., Tran, G., Heuzé, V. & Ankers, P. 2014. State of the art on use of insects as animal feed. *Anim. Feed Sci. Technol*. **197**, 1–33.

Marono, S., Loponte, R., Lombardi, P., Vassalotti, G., Pero, M. E., Russo, F., Gasco, L., Parisi, G., Piccolo, G., Nizza, S., Di Meo, C., Attia, Y.A. & Bovera, F. 2017. Productive performance and blood profiles of laying hens fed *Hermetia illucens* larvae meal as total replacement of soybean meal from 24 to 45 weeks of age. *Poult. Sci.* **96**, 1783–1790.

Nadtochii, L.A., Istomin, A.I., Melchakov, R.M., Muradova, M.B. & Zhavoronkova, A.S. 2017. Assessment of the high-protein medicine ‘Zooprotein’ as cat food. *International Research Journal* **63**, 49–52. (in Russian).

NRC. 2006. Energy. In: National Research Council, editor. Nutrient requirements of dogs and cats. Washingtion DC: *National Academic Press*. 22–48 pp.

Ramos, E.J., Gonzalez, E.A., Hernandez, A.R. & Pino, J.M. 2002. Use of Tenebrio molitor (*Coleoptera: Tenebrionidae*) to recycle organic wastes and as feed for broiler chickens. *Journal of Economic Entomology* **95**, 214–220.

Raubenheimer, D. & Rothman, J.M. 2011. Nutritional ecology of entomophagy in humans and other primates. *Annual Review of Entomology* **58**, 141–60.

Regulation (EC) No 999/2001 – rules for the prevention, control and eradication of certain transmissible spongiform encephalopathies **147**, 1.

Rumpold, B.A. & Schluter, O.K. 2013a. Nutritional composition and safety aspects of edible insects. *Molecular Nutrition and Food Research* **57**, 802–23.

Rumpold, B.A. & Schluter, O.K. 2013b. Potential and challenges of insects as an innovative source for food and feed production. *Innovative Food Science and Emerging Technologies* **17**, 1–11.

Rumpold, B. & Schluter, O. 2015. Insect-based protein sources and their potential for human consumption: Nutritional composition and processing. *Animal Frontiers* **5**, 20–24.

Salomone, R., Saija, G., Mondello, G., Giannetto, A., Fasulo, S. & Savastano, D. 2017. Environmental impact of food waste bioconversion by insects: Application of Life Cycle Assessment to process using *Hermetia illucens*. *J. Clean. Prod.* **140**, 890–905.

Schiavone, A., Cullere, M. & De Marco, M. 2017. Partial or total replacement of soybean oil by black soldier fly larvae (*Hermetia illucens L.)* fat in broiler diets: effect on growth performances, feed-choice, blood traits. *Italian Journal of Animal Science* **16**, 93–100.

Sencic, D., Samac, D., Antunovic, Z., Novoselec, J. & Klaric, I. 2011. Influence of crude protein level in forage mixture-son pig meat and carcass quality. *Macedonian Journal of Animal Science* **1**, 89–93.

Suchkova, E., Shershenkov, B. & Baranenko, D. 2014. Effect of ultrasonic treatment on metabolic activity of Propionibacterium shermanii, cultivated in nutrient medium based on milk whey. *Agronomy Research* **12**(3), 813–820.

Surendra, K.C., Olivier, R., Tomberlin, J.K., Jha, R. &. Khanal, S.K. 2016. Bioconversion of organic wastes into biodiesel and animal feed via insect farming. *Renew. Energy* **98**, 197–202.

Teymurazov, M.G., Svetoch, E.A., Manzenyuk, O.Y., Tazina, O.I., Fedorov, T.V., Istomin, I.I., Istomin, A.I. & Yakovlev, S.S. 2018. Antimicrobial activity of *Lucilia Caesar* larvae against bacteria, pathogenic to humans and birds. *Veterinary Medicine* **9**, 5 pp. (in Russian).

The European pet food industry (FEDIAF). Nutritional Guidelines for Complete and Complementary Pet Food for Cats and Dogs, 2014. Available at: http://www.fediaf.org/self-regulation/nutrition/

The European pet food industry (FEDIAF). Nutritional Guidelines for Complete and Complementary Pet Food for Cats and Dogs, 2017. Available at: http://www.fediaf.org/

Tilman, D. & Clark, M. 2014. Global diets link environmental sustainability and human health. *Nature* **22**, 515–518.

Van, H.A. 2013. Potential of Insects as Food and Feed in Assuring Food Security. *Annu. Rev. Entomol*. **1**, 563–583.

Van, H.A., Dicke, M. & Loon, J. 2015. Insects to feed the world. *J. Insects Food Feed* **1**, 3–5.

Wang, L., Li, J. & Jin, J.N. 2017. A comprehensive evaluation of replacing fishmeal with housefly (*Musca domestica*) maggot meal in the diet of Nile tilapia (*Oreochromis niloticus*): growth performance. *Aquaculture Nutrition* **23**, 983–93.

Yasir, A.B., Abdul, M.G. & Haidar, A.A. 2017. Prospects of complete feed system in ruminant feeding: A review. *Vet. World* **10**, 424–437.

Zanten, H.E, Mollenhorst, H., Klootwijk, C.W., Middelaar, C.E. & de Boer, I.J.M. 2016. Global food supply: Land use efficiency of livestock systems. *Int. J. Life Cycle Assess*. **21**, 747–758.

Agronomy Research **17**(6), 2373–2385, 2019
https://doi.org/10.15159/AR.19.210

Short-term effect of sawdust biochar and bovine manure on the physiological behavior of turnip (*Brassica rapa* L.) grown in open fields in the Algiers region

S. Nouar[1,*], M. Baha[2], M. Latati[1], R. Djebbar[3] and L. Reguieg[1]

[1]National School of Agronomy, Department of Plant Productions. Laboratory of Integrated Improvement of Agricultural Productions, Laboratory of Plant Production. Rue Hassen Badi, El Harrach DZ16200 Algiers, Algeria
[2]National School of Higher Education (ENS), Department of General Agronomy. Laboratory of Ecobiology animal. Post box 92 old Kouba1 DZ6308 Algiers, Algeria
[3]Université des Sciences et Technologies Houari Boumediene Faculty of Biological Sciences, Laboratory of Biology and Physiology of Organisms. Mailbox 32 El Alia Bab ezzouar DZ16111 Algiers, Algeria
[*]Correspondence: samnour207@gmail.com

Abstract. This study was designed to determine the effect of different doses of biochar (B) 5.10, 20 t ha^{-1} alone and mixed with manure (F) 10 t ha^{-1} on turnips. The results showed that the OM (organic matter) rate had a maximum of 93.7% for (B20*F) and a minimum of 14.5% for (F); the CEC (cation exchange capacity) showed a maximum of 32.2% for (B10*F) and a minimum of 0.2% with (B5*F) compared to the control (T) and finally the pH to be increased with a maximum value of 11.2% for (B20*F) and a minimum value of 1.7% for (F) compared to (T) (≤ 0.01).
For the chemical parameters of the turnip, the maximum nitrogen rate was 93.8% with (B10) and 2% for (B20). The highest value for phosphorus was recorded in (F) and a minimal value in (B5) (≤ 0.01). The potassium level was high 4.2% for the treatment (B20*F) with the lowest value of 4.4% for (B5) and (B10) compared to (T) (0.05).
For the yield components, the fresh weight of the most important bulb was obtained with (F) with the value of 116.8% and minimum weight of 0.4% in the treatment (B5). The highest bulb length value was 36.8% in (F) and the lowest was 0.5% obtained with (B20*F). The bulb diameter was the largest in the treatment (F) and the smallest was 4.8% in (B20). Finally, the fresh weight of the leaves showed a maximum of 106.9% in (F) and an increase of 6% in (B20) compared to (T) (≤ 0.01).

Key words: *Brassica rapa* L, bulb nutrients, wood biochar, cattle manure, soil.

INTRODUCTION

The progress made in crop intensification through the use of mineral fertilizers, new cultivation techniques, variety creations, etc., has not led to an increase in agricultural production. Yields of the main crops remain in decline due to soil depletion through leaching of mineral elements resulting in their depletion in organic matter (OM).

In the current context where environmentally friendly approaches are increasingly valued, global choices are moving towards sustainable agriculture that relies on the use of fewer chemical inputs and promotes the use of organic products that play an important role in addressing various environmental issues. Indeed, the conservation of the fertility of cultivable soils is one of the challenges that is becoming essential for obtaining satisfactory yields in terms of quality and quantity (Lehmann et al., 2006a ; Latati et al., 2016).

Soil organic carbon content plays an important role in soil fertility and is considered the main indicator of soil quality (Lehmann, 2007; Spokas et al., 2012). A loss of organic matter directly results in a decrease in their value, creating a vicious circle: soil degradation, lower yields, use of food imports, food insecurity, malnutrition and famine in some countries (Laird et al., 2010). In recent years, much progress has been made in understanding the impact of a 'biochar' amendment on soil carbon dynamics.

Indeed, burying bio-tanks in the soil could become one of the major ecological intensification techniques for 21st century agriculture. The usefulness of its application in soils has been deduced from observations made on black lands in the Amazon: Terra Preta, which have shown an improvement in physico-chemical and biological properties (Lehmann et al., 2006b; Lehmann, 2007).

The availability of nutrients for plants is becoming one of the obstacles to increasing crop production. Indeed, these soil nutrients are provided as fertilizers, some will be used by plants, some will be fixed by soil colloids and the rest will be lost through volatilization or leaching. Lehmann et al. (2011) reported that biochar can fix the nutrient content in the soil to limit these different forms of losses.

The biochar is one of the natural products that can answer this problem. It results from the pyrolysis of biomass under a relatively high temperature ≤ 700 °C and a limited supply of oxygen. It is not only a fertilizer, but also it is mainly a vector for the diffusion of nutrients and a habitat for the microorganisms due its high porosity and delete specific surface. Moreover increases the capacity of water retention of the grounds (Lehmann et al., 2011). Indeed, biochars are characterized by a strong cation exchange capacity (CEC), significantly improve the nutrition of plants and with its low density it confers a good root development (Glaser et al., 2002; Zhang et al., 2010). It is very recalcitrant to microbial decomposition and thus guarantees mediu²m and long term sequestration of organic carbon in order to make it available over time to the plant especially in degrading soils and even in temperate regions. Recent studies have shown that it is also of interest against global warming and reduces greenhouse gas emissions (Lehmann et al., 2006b).

In Algeria, the level of organic matter remains low (an average of 1.2%) because of inadequate, poorly exploited, cultivation techniques adopted such as plowing, non-compliance with crop calendars in some regions. The main question of our research is: how can we contribute to improving our yields by increasing the fertility of our soils ? so as to reduce our imports of fertilizers and/or foodstuffs in order finally to be able to ensure a certain food safety while preserving the environment.

It should be noted that the impacts of bio-tanks could vary depending on their nature, pyrolysis process and the soils to which they will be applied (Lehmann et al., 2006a; Spokas & Reicosky, 2009; Ippolito et al., 2012). We proposed to introduce it by combining it with cattle manure in our experiment to stimulate microbial life, increasing the availability of nutrients for turnips and enriching the soil with carbon. It should be noted that the impacts of biochars may vary according to their nature, pyrolysis process and soil types (Lehmann et al., 2006a; Spokas & Reicosky., 2009).

This study aimed to evaluate the effect of three doses of wood biochar and a single dose of cattle manure on certain physico-chemical properties of our soil and certain parameters of turnip growth, in order to determine if it is conceivable to introduce it into current farming practices as part of a perspective of contributing to the improvement of crop yields, in order to provide farmers with a sustainable and profitable farming system capable of improve the fertility of their soil and preserve the environment (limit the pollution of groundwater ....).

MATERIALS AND METHODS

Experimental site

Our experiment was conducted in the field during the 2015/2016 agricultural campaign at the experimental station of ENSA (El Harrach National Agronomic School) which is located in the North East of Algiers, located between latitude: 36° 43'North longitude: 30° 8' East altitude: 50 m bioclimatic floor: sub-humid to mild winter. The previous crop was a bean crop (*Vicia faba major* L).

Our experiment consisted of the study as follows

1-The biochar with the following doses: 5 t ha^{-1}, 10 t ha^{-1} and 20 t ha^{-1}

2-Manure with a dose of 10 t ha^{-1}

3- Witness (T)

The eight treatments were randomly organized with a device in total randomization with five blocks (repetitions). Each block consists of 8 plots of 1×1 m each. The blocks were spaced one meter apart and the plots were 0.5 m apart.

The different combinations are as follows:

T1 : B5	→ (5 t ha^{-1})	T5 : B20	→ (20 t ha^{-1})
T2 : B5*F	→ (5 t ha^{-1}+10 t ha^{-1})	T6 : B20*F	→ (20 t ha^{-1}+10 t ha^{-1})
T3 : B10	→ (10 t ha^{-1})	T7 : F	→ (10 t ha^{-1})
T4 : B10*F	→ (10 t ha^{-1}+10 t ha^{-1})	T8 :	→ witness

B : biochar

F : manure

According to a large number of bibliographic references (Laird et al., 2010 & Tammeorg et al., 2014), the long-term maintenance of soil fertility formerly amended in biochar compared to a growing number of trials in many countries, shows that the introduction of 5 t ha^{-1} to 20 t ha^{-1} of biochar per hectare can double productivity and create long-term fertility in the field from which the choice of these doses was proposed.

Incorporation of wood biochar and bovine manure

The biochar used in the experiment was obtained from a sawdust with a traditional method, which then sieved to 2 mm before application.

The manure used for our test was obtained from our school's central farm (ENSA), let it decay a few months. After soil preparation (plowing and harrowing). The quantities of biochar and manure from the different treatments were weighed and distributed evenly over the different micro-plots according to the conditions required on the soil. The incorporation of the two amendments was done manually at a depth of about 10 cm

2 weeks before sowing turnip seeds.Turnip *(Brassica rapa* L.) variety 'hammer', grown for its large, swiveling, bulbous and succulent root (ITCMI, 2010) was sown on 17 January 2016. Direct seeding was conducted in-line with spacing between rows of 20 cm and 15 cm between plants. Thinning was done afterwards; weeding was done on a weekly basis and manually; Irrigation was applied throughout the experiment.

The physical and chemical properties of the soil

A diagonal soil sample was taken before the crop was planted at a depth of about 20 cm approximately.The collected soil samples pooled, air-dried and sieved with a 2 mm sieve and analyzed for nitrogen, phosphorus, potassium, CEC, particle size, organic matter, and pH.

– Organic carbon (Matieu & Pieltain, 2003).
– The pH with distilled water (2v/5v) (Baize, 1988).
– The salinité with distilled water (1v/5v) (Baize, 1988).
– Total nitrogen by Kjeldahl method (Matieu & Pieltain, 2003).
– Exchangeable potassium extracted using 1M ammonium acetate (flame spectrophotometer) (Baize, 1988).
– The assimilable phosphorus (Olsen et al., 1954).
– The CEC carried out with sodium acetate and potassium acetate (Matieu & Pieltain, 2003).

The total limestone measured with Bernard calcimeter (Matieu & Pieltain, 2003).

The picking of the fruit was done manually by hand, once uprooted the tubers are washed in tap water to remove any trace of soil then weighed on a precision scale to determine their fresh weight. The length was measured using a ribbon meter and the diameter using a vernier caliper.

Analysis of wood biochar and bovine manure

– The chemical composition of our biochar and bovine manure used was analyzed after drying then grinding and sieving to be ready for analysis.The organic carbon content of the substrates was measured by calcination in the calcination furnace (30 minutes at 500 °C and then 6 hours at 600 °C).

During the harvest of the turnip in 2015/2016, five samples are taken per micro-parcel weighed, washed then dried at 60–70 °C for 48 h and finally milled in a vegetable mill for the analyzes which are:

– Total nitrogen (Kjeldahl method) (Matieu & Pieltain, 2003).
– Vegetable phosphorus revealed by the vanadomolibdique reagent (AFNOR, 1969).
– Vegetable potassium extracted with 0.1N nitric acid (El mekaoui, 1987).

Statistical analysis

The data collected for each experiment was analyzed by the Statistical Package Version 8 (ANOVA) and the treatment facilities were compared using the Tukey Test at probability level $p = 0.05$.

RESULTS AND DISCUSSION

Chemical analyses of the soil before seeding and of manure and biochar are illustrated in Tables 1 and 2. The soil of our experimental site is of a fine-argillaceous silt texture, neutral at low organic matter content, quite rich in total nitrogen, low rate of assimilable phosphorus, moderately rich in exchangeable potassium.

The pH of the biochar was alkaline, rich in organic carbon and poor in total nitrogen resulting in a high content of C/N, a low level of available phosphorus but sufficiently rich in exchangeable potassium. The manure was characterized by a neutral pH, with higher concentrations of total nitrogen, available phosphorus and exchangeable potassium compared to biochar.

Table 1. Soil properties of the site before experimentation

Property	Value
pH (water)	7.1
N Total ($NH^{4+)}$ ppm	0.87
$CaCO_3$%	1.03
k^+ (meq100^{-1}g soil)	0.45
p (ppm)	5.98
CEC (cmol kg^{-1} soil)	4.19
C %	0.37
OM %	0.63
N assimilable (ppm)	12.05
EC 10^{-3}ms cm^{-1}	123.44
Sand (%)	17.22
Silt (%)	54.55
Clay (%)	28.23
Textural class	Loam clay

Table 2. Chemical composition of biochar and manure

Property	Biochar	manure
pH	8.29	7.4
N Total (NH^{4+}) (%)	0.28	0.61
C Pendéral (%)	93.5	23
K^+ (meq 100g^{-1} soil)	0.51	0.81
P (ppm)	2.01	40.79
CEC (cmol kg^{-1} soil)	3.3	5.28

The use of biochar and manure alone and in mixture generally caused an increase in the soil component ie organic carbon and CEC relative to witness. Indeed, (Table 3) shows the effect of bovine manure alone on the soil chemical properties. During this test, the manure individually slightly increased the rate of some soil elements studied as organic matter and CEC relative to the control in a very highly significant ($p \leq 0.001$) with a rate of 1.1% and of 4.35 cmol kg^{-1} soil respectively. In addition, the application of different doses of Biochar individually increased in a very highly significant ($p \leq 0.001$), soil pH values increased by an average of about 1.2 units compared to the control. The organic matter showed a very highly significant difference ($p \leq 0.001$) with a maximum of 1.64% compared to the control and a maximum value of 5.39 cmol kg^{-1} for the CEC.

(Table 3) shows the effect of mixing manure with biochar on the soil chemical properties.

For the soil parameters, statistical analysis showed that there were very significant differences between the tratements, particularly for pH. Indeed, (B20) and (B20*F) gave the highest pH compared to the other treatments, the (F) and (T) in last position ($p \leq 0.001$).

During this test, for organic matter the results showed that the best results were obtained with (B10), (B10*F), (B20) and (B20*F) treatments compared to other treatments. For the CEC, the results showed that these same treatments have given the best results ($p \leq 0.001$).

Table 3. Effect of biochar and manure on soil

± Treatment	PH	OM (%)	CEC (cmol per kg soil)
B5	7.74 ± 0.03 [c]	1.05 ± 0.05 [b]	4.04 ± 0.14 [b]
B5*F	7.61 ± 0.03 [c]	1.12 ± 0.02 [b]	4.20 ± 0.12 [b]
B10	8.16 ± 0.01[b]	1.61 ± 0.15 [a]	5.24 ± 0.23 [a]
B10*F	8.15 ± 0.01[b]	1.73 ± 0.22 [a]	5.55 ± 0.18 [a]
B20	8.28 ± 0.00 [a]	1.64 ± 0.1 [a]	5.39 ± 0.2 [a]
B20*F	8.28 ± 0.01 [a]	1.86 ± 0.13 [a]	5.53 ± 0.22 [a]
F	7.31 ± 0.03 [d]	0.97 ± 0.02 [b]	4.35 ± 0.24 [b]
T	7.44 ± 0.03 [d]	1.07 ± 0.02 [b]	4.2 ± 0.16 [b]
p value	≤ 0.001	≤ 0.001	≤ 0.001

F: manure; B: biochar with B5: 5 t ha^{-1}, B10: 10 t ha^{-1}, B20: 20 t ha^{-1}; OM: organic matter; CEC: cation exchange capacity; Means with the different letter are highly significantly different acordind to the Tukey's test ($P \leq 0.001$) Value with ± represent the standard errors.

Results on the effect of Biochar and manure on the nutrient concentration of the turnip tuber are presented in (Table 4). In general, treatment of manure alone influenced very highly ($p \leq 0.001$) and significantly increased the concentrations of nitrogen, phosphorus ($p \leq 0.001$) and potassium ($p \leq 0.05$) in tissues turnip tuber.

Table 4. Effect of Biochar and manure on the chemical elements of the turnip bulb

± Treatment	P (P mg per 100g ps)	N (%)	K (k^+mg per 100 g ps)
B5	302.69 ± 17.13 [bc]	0.44 ± 0.04 [d]	$2{,}866.66 \pm 169.97$ [c]
B5*F	372.37 ± 28.09 [ab]	0.65 ± 0.02 [bc]	$3{,}666.66 \pm 182.57$ [b]
B10	258.38 ± 2.58 [cd]	0.95 ± 0.06 [a]	$3{,}133.33 \pm 133.33$ [bc]
B10*F	298.46 ± 5.09 [c]	0.75 ± 0.06 [ab]	$3{,}933.33 \pm 286.74$ [a]
B20	233.41 ± 7.57 [d]	0.50 ± 0.03 [cd]	$3{,}266.66 \pm 323.18$ [bc]
B20*F	227.26 ± 7.61 [d]	0.55 ± 0.04 [cd]	$4{,}266.66 \pm 476.1$ [a]
F	436.22 ± 19.10 [a]	0.81 ± 0.03 [ab]	$3{,}866.66 \pm 583.1$ [b]
T	315.25 ± 22.86 [ab]	0.5 ± 0.04 [cd]	$2{,}999.99 \pm 105.41$ [c]
p value	≤ 0.001	≤ 0.001	≤ 0.05

F: manure; B: biochar with B5: 5 t ha^{-1}, B10: 10 t ha^{-1}, B20: 20 t ha^{-1}; P: phosphorus; N: nitogen; K: potassium; PS: dry weight; Means with the different letter are highly significantly and significantly different acordind to the Tukey's test ($P \leq 0.001$) and ($P \leq 0.05$) Value with ± represent the standard errors.

In this test, the effect of the biochar applied alone and mixed with manure had a very highly significant effect. For phosphorus, the results showed very highly significant differences ($p \leq 0.001$). Indeed, it decreased significantly for the dose of (B20*F) and (B20). The manure (F) was distinguished from other treatments by ranking first with the best phosphorus concentrations compared to other treatments.

For the total nitrogen, the results showed that for all the different doses applied, the effect was significant ($p \leq 0.01$) compared to the control with (B10) which gave the best results and led the groups. (B5) is in last position with the lowest nitrogen concentration.

For potassium, the results showed that for all the different doses applied the effect was significant ($p \leq 0.05$) compared to the control in the tissues of the turnip tuber with (B20*F) at the top of the ranking.

Our results revealed an interactive effect between the different doses of Biochar and the manure dose, which were very highly significant ($p \leq 0.001$) for all the chemical elements studied, namely nitrogen and phosphorus, and significant ($p \leq 0.05$) for potassium.

Results on the effect of Biochar and manure on turnip yield components are as follows:

Presented in (Table 5); when wood biochar and manure are studied on biometric parameters related to turnip yield; bovine manure was found to be very highly ($p \leq 0.001$) increased fresh weight bulbs, diameter, bulb length and fresh weight leaves with the highest values with the best yield compared to all other treatments.

Table 5. Effect of biochar and manure on turnip yield components

± Treatment	FWB (g)	Diameter (cm)	BL (cm)	FWL (g)
B5	59.00 ± 4.43 ^{cd}	4.24 ± 0.22 ^{b}	8.97 ± 0.5 ^{c}	12.47 ± 1.78 ^{bc}
B5*F	97.80 ± 9.15 ^{b}	4.62 ± 0.19 ^{ab}	11.89 ± 0.45 ^{a}	22.08 ± 5.17 ^{ab}
B10	81.76 ± 7.22 ^{bc}	4.42 ± 0.16 ^{ab}	11.12 ± 0.65 ^{ab}	14.42 ± 1.90 ^{abc}
B10*F	86.36 ± 4.90 ^{b}	4.47 ± 0.19 ^{ab}	11.40 ± 0.23 ^{a}	15.68 ± 1.51 ^{abc}
B20	50.24 ± 5.04 ^{d}	4.08 ± 0.32 ^{b}	8.83 ± 0.40 ^{c}	10.52 ± 1.63 ^{c}
B20*F	56.52 ± 3.65 ^{cd}	4.27 ± 0.20 ^{b}	9.37 ± 0.38 ^{b}	12.3 ± 1.09 ^{bc}
F	127.30 ± 5.24 ^{a}	5.24 ± 0.1 ^{a}	12.75 ± 0.32 ^{a}	23.19 ± 1.38 ^{a}
T	58.72 ± 2.54 ^{cd}	3.89 ± 0.1 ^{b}	9.32 ± 0.31 ^{b}	11.20 ± 1.15 ^{c}
p value	≤ 0.001	≤ 0.001	≤ 0.001	≤ 0.001

F: manure; B: biochar with B5: 5 t ha^{-1}, B10: 10 t ha^{-1}, B20: 2 0t ha^{-1}; FWB: Fresh weight bulbs; D: Diameter; BL: Bulb length; FWL: Fresh weight leaves Means with the different letter are highly significantly different acordind to the Tukey's test ($P \leq 0.001$) Value with ± represent the standard errors.

nb: these results are similar to those of another potted trial carried out with a sandy soil not yet published and with the same variety of turnip (hammer) where the yield components were low for high doses in biochar alone and mixed with manure; the best results were obtained with the manure dose alone.

Biochar vary greatly in composition, pH and physical properties (Chan et al., 2007; Lehmann., 2007). Before applying them, it is important to note that during pyrolysis all the nitrogen contained in the original biomass is either trapped in the aromatic structure or volatilized (Steiner et al., 2010). The addition of 'fresh' biochar alone to soils often results in what is called a 'nitrogen shock', limiting its bioavailability for plants (Alexis et al., 2007; Chan et al., 2008; Sanchez-Garcia et al., 2015), hence the need to associate a nitrogen source such as manure with it to avoid immobilization. Our results showed that the values of the different treatments, namely (B20*F), (B20) and (B5) of biochar alone, were close to the control, with the exception of the treatment (B10) and (F), which showed the highest values compared to the control and the remains of the treatments.

According to Lehmann et al. (2003); Chan et al. (2008); Steiner et al. (2010); Steiner et al. (2010) biochar reduces nitrogen losses in the soil by leaching, so it is conducive to better soil fertility and reduced environmental impacts (groundwater pollution); also Laird et al. (2010); Zheng (2013) noted a significant 30% reduction in NO_3^- losses by leaching into amended soils into biochar, resulting in better nitrogen availability. This could explain the increase in nitrogen rate for treatments (B10) and (B10*F) compared to other treatments.

In a laboratory study where biochar was applied without manure (Ippolito et al., 2014) showed that the application rate of 10% biochar significantly reduced NO_3^- concentrations in the soil. While Foster et al. (2016) demonstrated that with a dose of 30 t ha^{-1} biochar, no decrease or increase in nitrogen in soil at alkaline pH in maize was observed.

The results of this test are consistent with those obtained by (Ippolito et al., 2016), where the joint application of the biochar-manure mixture to limestone soil at different rates resulted in beneficial effects on soil properties. In another field study, the application of biochar and manure at doses of 22.4 and 42 t ha^{-1} respectively resulted in a decrease in NO_3^- content in the soil during the first year at alkaline pH (Ippolito et al., 2016). According to Lentz & Ippolito (2012), over time soil NO_3^- increases with all applied biochar rates when mixed with 2% manure, probably due to manure mineralization and nitrification.

The treatment deferents increased the soil pH except for manure and control, which created an unfavourable environment for the development of our crop, knowing that the optimal pH of turnip is 6–6.5 (ITCMI, 2010). The soil pH increased from about 1.0 to 1.4 units for all treatments except (F) and the control, this could surely be related to the alkaline effect of our biochar because of its chemical composition (related to the liming of the soil).

Generally, bacteria and fungi respond differently to a change in pH in the soil, microbial biomass increases with a pH increase from 3.7 to 8.3 (Novak et al., 2012). Indeed, bacteria respond positively to a pH increase above 7 while fungi show no significant change in their total biomass (Steinbeiss et al., 2009). Thus, microbial abundance would be influenced by adhesion to the biochar (Glaser et al., 2002; Steinbeiss et al., 2009). This suggests that increasing the pH creates a favourable environment for the survival and development of soil fauna. According to DeLuca et al. (2009), the alkaline pH of soils is due to the ash and carboxylic groups contained in the biochar, depending on the nature of the biomass treated.

Concerning phosphorus, this element plays a major role in root development, leading to an increase in tomato fruit production (Hossain et al., 2010). Note that phosphorus is blocked at pH below 6 and above 7.5, it is bioavailable at pHs between 6 and 7.5 (Zhang et al., 2010; Ippolito et al., 2014; Houben et al., 2014). In addition, when Ca^{2+} is in excess in the soil, phosphorus release is reduced due to the formation of poorly soluble phosphates (Nelson et al., 2011; Foster et al., 2016). This could explain the low phosphorus levels observed in turnip tissues obtained with almost all treatments except (F) compared to the control and also the low concentration of phosphorus in soil and biochar. The general trend shows a significant decrease in tuber size (yield), so it is difficult to conclude on the ‘fertilizing’ effect of biochar alone in turnips under our conditions.

In temperate systems, field trials without phosphorus fertilization have shown that pine biochar has decreased the available phosphorus in the soil, this decrease has been attributed to soil alkalinization (Nelson et al., 2011). According to Laird et al. (2010); Foster et al. (2016), the fixation of phosphorus by calcium and its adsorption on biochar surfaces has made it less available to the plant. Xu et al. (2014); Foster et al. (2016); Ige et al. (2005) demonstrated that there was a significant decrease in phosphatase activity in maize and hypothesized that pine biochar interacted with the production signals of this enzyme. They also reported that the effect of biochar on phosphorus adsorption is highly influenced by soil pH. Indeed, at a pH around 7–7.5, phosphorus is fixed by calcium and thus becomes less available, especially when calcium is in excess in the soil, its release is reduced because of the formation of poorly soluble phosphates. Thus, the fixation of phosphorus in soils amended with biochar can therefore be explained by their high calcium content.

Concerning potassium, the losses of this element by leaching seem to be less significant in the presence of biochar compared to the control because of its presence in turnip tissues, particularly for treatments (B10*F) and (B20*F).

There was an increase in potassium levels in our turnips as a result of the application of biochar or manure. This increase was greater with the mixing of the two compared to the control. This could be related to the presence of potassium in both amendments and in our soil for almost all the treatments studied. This can be explained by the fact that adding manure to biochar can increase oxidation of the biochar surface under favourable conditions (sufficient moisture, favourable temperature), to release existing potassium and make it available to the turnip (Adekiyaa, et al., 2016; Biederman et al., 2017).

Research has shown that applying biochar to the soil can improve the cation exchange capacity (CEC) of soils, resulting in reduced nutrient leaching and thus improved availability (Liang et al., 2006). Indeed, biochar has a porous structure with a large exchange surface and a negative surface charge, giving it a high capacity for cation exchange in the soil for good nutrient retention (Zhang, et al., 2014). This CEC would likely increase over time due to aging and incubation of the biochar in the soil, oxidation of its surfaces and/or adsorption of organic acids (Spokas & Reicosky, 2009). Indeed, just like the chemically active colloidal fraction of soils, over time it can begin to show a larger loading surface and can therefore increase the CEC of soils (Laird et al., 2010).

Regarding the comparison between treatments, the results showed an improvement in CEC compared to the control, especially with the treatment (B10), (B20), (B10*F) and (B20*F). The very low CEC of our soil is an indication that it contains low nutrient reserves. This could be related to soil mineralogy, including the very low clay fraction (Liang et al., 2006; Major et al., 2012) and the low organic matter content.

The decreases in mineral elements observed in the control are mainly due to the reduction in the soil carbon content. Indeed, organic matter is directly related to soil CEC; this has been highlighted by several authors (Lehman et al., 2006a; Atkinson et al., 2010; Laird et al., 2010; Zhang et al., 2010).

For organic carbon, it increased significantly with a biochar amendment (B10) and (B20) compared to (F) and the control confirming its sequestration potential. These results are consistent with those of Adekiyaa et al. (2019) and Foster et al. (2016). Although it is resistant to microbial decomposition, it guarantees long-term soil fertility (Steiner et al., 2007; Sohi, 2012; Zhang, 2014). According to Steinbeiss et al. (2009), the nature of biochar has a real influence on soil organic carbon losses, its measurement makes it possible to monitor its evolution with different amendments in different soil types.

The highest organic matter rate obtained is 1.86% with the treatment (B20*F). Although our soil is poor in organic matter and even after organic matter inputs, our results remain low compared to other work. This may be due to the recalcitrance of the biochar (Lehmann et al., 2003), or may be related to the analytical method used because according to (Blanc, 2013), it is likely that the calcination method detects about 70% organic matter in a pure biochar while the potassium dichromate method only 6%. In addition, potassium dichromate reacts more with fine biochar particles. This is certainly related to the much larger attack surface for fine particles than for coarse particles, whereas with the calcination method, coarse particles have a higher organic matter content than fine particles; organic matter analyses are therefore biased for samples containing biochar.

The application of biochar alone or mixed with manure had a negative influence on turnip yield (low weight stunted tubers) especially at high doses of (B20) and (B20*F) compared to the control and treatment remains. This could be explained by its nature (high pH,...) necessary for root development, knowing that turnips prefer humus-bearing, slightly acidic or neutral soils with an optimal pH (6–6.8), they have difficulty tolerating alkaline soils, which is the case for most soils in temperate regions (Lentz & Ippolito, 2012). On the other hand, biochar improves penetration depth by its ability to reduce soil compaction (Glaser et al., 2002; Atkinson et al., 2010), this has been demonstrated in asparagus where the number of roots has increased with the addition of coconut biochar in tropical soil (Matsubara et al., 2002; Lehman et al., 2011); also, in rice where root length has increased with biochar additions (Noguera et al., 2010; Lehman et al., 2011).

According to our study, the contribution of manure alone did not increase organic carbon and soil CEC like biochar, however the improvement in turnip performance by manure was attributed to its neutral pH, low C/N content, richness in minerals available to our crop that increased tuber size and weight compared to the control and biochar alone. Indeed, the manure supply significantly improved the quality of the fruit in the treatments amended to manure alone or in a mixture for treatment (B5*F) compared to the control and the remains of the treatments. This result can be explained by better soil biological activity due to an ideal pH for good mineralization of organic matter, thus allowing better bioavailability of mineral elements for turnips, resulting in acceptable fruit quality contributing to satisfactory yields (Biederman et al., 2017; Adekiyaa et al., 2019).

Our results are consistent with those of Foster et al. (2016) and Ippolito et al. (2016) who hypothesized that relatively low biochar application rates would not cause a negative effect while excessive biochar applications (10% and more) would cause a negative priming effect even if in the presence of manure this remains related to each crop and soil type.

CONCLUSION

This trial found that, in the short term, the addition of wood biochar and bovine manure alone or as a mixture to the soil helped to improve some soil parameters such as CEC and organic matter.Although biochar has several benefits to our soil that can be appreciated in bulb vegetables and that have a direct impact on their growth and development because of the improvement in their depth of penetration by its ability to reduce the soil compaction by increasing porosity resulting in better water retention and nutrients.

Overall, the development of sustainable agriculture involves the identification and development of new strategies to maintain long-term returns. In the future, biochar could be associated with manure or other organic sourcesfrom farms or agro-food industries or to another inorganic one, which could be one of the possible future solutions for the sequestration of organic carbon in degraded soils (Lehmann, 2007; Sohi, 2012). Indeed, it is important to mention that the effects of biochar can not be generalized since they are specific to the type of biochar to use, to the selected crop, nature of the soil and to the technical route to adopt; the ideal would be to establish a rate for each soil and for each plant species because it is not the contribution of carbon as such which is important for soil fertility, but rather the monitoring of its decomposition and its impact on the agro system.

REFERENCES

Adekiyaa, A.O., Agbedeb, T.M., Aboyejia, C.M., Dunsina, O. & Simeona, V.T. 2019. Effects of biochar and poultry manure on soil characteristics and the yield of radish. *Scientia Horticulturae* **243**, 457–463.

Afnor. 1969. *Spectrophotometric determination of phosphoric anhydrine using the vanadomolybdic method* (*Dosage spectrophotométrique de l'anhydrine phosphorique méthode vanadomolybdique*). Paris, 255 pp.

Alexis, M.A., Rasse, D.P., Rumpel, C., Bardoux, G., Pechot, N., Schmalzer, P., Drake, B. & Mariotti, A. 2007. Fire impact on Cand N losses and charcoal production in a scrub oak ecosystem. *Biogeochemistry* **82**, 201–216.

Atkinson, C.J., Fitzgerald, J.D. & Hipps, N.A. 2010. Potential mechanisms for achieving agricultural benefits from biochar application to temperate soils. *Plant and Soil*. **337**, 1–18.

Baize, D. 1988. *A guide to current soil analysis (Guide des analyses courantes en pédologie)*. INRA, Paris, 172 pp.

Biederman, L.A., Phelps, J., Ross, B.J., Polzin, M. & Harpole, W.S. 2017. Biochar and manure alter few aspects of prairie development. A fie.ld test. *Agriculture, Ecosystems and Environment* **23**, 78–87.

Blanc, A. 2013. Propriétés physico-chimiques d'un sol amendé en biochar. Genève, Novembre 120 p. https://docplayer.fr

Chan, K.Y., van Zwieten, L., Meszaros, I., Downie, A.& Joseph, S. 2007. Agronomic values of green waste biochar as a soil amendment. *Aust. J. Soil Res*. **45**, 629–634.

Chan, K.Y., van Zwieten, L., Meszaros, I., Downie, A. & Joseph, S. 2008. Using poultry litter biochars as soil amendments. *Aust. J. Soil Res*. **46**, 437–444.

DeLuca, T.H., MacKenzie, M.D. & Gundale, M.J. 2009. Biochar effects on soil nutrient transformations. In: Lehmann, J., Joseph, S. (Eds.), *Biochar for Environmental Management. Science and Technology*. Earthscan, London. pp. 251–270.

El Mekaoui, M. 1987. Contribution to the study of salinity tolerance in durum wheat (*Triticum durum* Desf.) and barley (*Hordeum vulgare* L.). (*Contribution à l'étude de la tolérance à la salinité chez le blé dur* (*Triticum durum* Desf.) et l'orge (*Hordeum vulgare* L.). Diploma from DEUA, Montpellier, France, 178 pp.

Foster, E.J., Neil, H., Wallenstein, M., Cotrufo, M. & Francesca. 2016. Biochar and manure amendments impact soil nutrients and microbial enzymatic activities in a semi-arid irrigated maize cropping system. *Agriculture Ecosystems and Environment* **233**, 404–414.

Glaser, B., Lehmann, J. & Zech, W. 2002. Ameliorating physical and chemical properties of highly weathered soils in the tropics with charcoal. *Biology and Fertility of Soils* **35**, 219–230.

Hossain, M.K., Strezov, V., Yin, C.K. & Nelson, P.F.2010. Agronomic properties of wastewater sludge biochar and bioavailability of metals in production of cherry tomato (*Lycopersicon esculentum*).*Chemosphere* **78**, 1167–1171.

Houben, D., Hardy, B., Faucon, M.P. & Cornelis, J-T. 2014. Biochar had effects on phosphorus sorption and desorption in three soils with differing acidity. *Ecological Engineering* **62**, 54–60.

Ige, D.V., Akinremi, O.O., Flaten, D.N., Ajiboye, B. & Kashem, M.A. 2005. Phosphorus sorption capacity of alkaline Manitoba soils and its relationship to soil properties. *Can. J. Soil Sci*. **85**, 417–426.

Ippolito, J.A., Laird, D.A. & Busscher, W.J. 2012. Environmental benefits of biochar. *J.Environ. Qual*. **41**, 967–972.

Ippolito, J.A., Stromberger, M.E., Lentz, R.D. & Dungan, R.S. 2014. Hardwood biochar influences calcareous soil physicochemical and microbiological status. *J.Environ.* **43** 681–689.

Ippolito, J.A., Stromberger, M.E., Lentz, R.D. & Dungan, R.S. 2016. Hardwood biochar and manure co-application to a calcareous soil. *Chemosphere* **142**, 84–91.

ITCMI. 2010. Fiches techniques valorisées des cultures maraîchères et Industrielles : La culture du NAVET 5p. Inter itcmi2008@yahoo.fr

Laird, D.A., Fleming, P.D., Wang, B., Horton, R. & Karlen, D.L. 2010. Biochar impact on nutrient leaching from a Midwestern agricultural soil. *Geoderma.* **158**, 436–442.

Laird, D.A., Fleming, P., Davis, D.D., Horton, R., Wang, B. & Karlen, D.L. 2010. Impact of biochar amendments on the quality of a typical Midwestern agricultural soil. *Geoderma* **158**, 443–449.

Latati, M., Bargaz, A., Belarbi, B., Lazali, M., Benlahrech, S., Tellah, S., Kaci, G., Drevon, J.J. & Ounane, S.M. 2016. The intercropping common bean with maize improves the rhizobial efficiency, resource use and grain yield under low phosphorus availability. *Eur. J. Agron*. **72**, 80–90.

Lehmann, J., da Silva, J.P., Steiner, C., Nehls, T., Zech, W. & Glaser, B. 2003. Nutrient availability and leaching in an archaeological Anthrosol and a Ferralsol of the Central Amazon basin: fertilizer, manure and charcoal amendments. *Plant Soil*. **249**, 343–357.

Lehmann, J., Gaunt, J. & Rondon, M. 2006a. Bio-char sequestration in terrestrial ecosystems. *Mitigation and Adaptation Strategies for Global Change* **11**, 403–427.

Lehmann, J., Gaunt, J. & Rondon, M. 2006b. Biochar sequestration in terrestrial ecosystems–A review. *Mitigation and Adaptation Strategies for Global Change* **11**, 395–419.

Lehmann, J. 2007. A handful of carbon. *Nature* **447**, 143–144.

Lehmann, J. & Stephen, J. 2009. *Biochar for environment management : science ans technology*. London, GB: Earthscan, 448 pp.

Lehmann, J., Rillig, M.C., Thies, J., Masiello, C.A., Hockaday, W.C. & Crowley, D. 2011. Biochar effects on soil biota—a review. *Soil Biol. Biochem*. **43**, 1812–1836.

Lentz, R.D. & Ippolito, J.A. 2012. Biochar and manure affects calcareous soil and corn silage nutrient concentrations and uptake. *J. Environ. Qual*. **41**,1033–1043.

Liang, B., Lehmann, J., Solomon, D., Kinyangi, J., Grossman, J., O'Neill, B., Skjemstad, J.O., Thies, J., Luiza, F.J., Petersen, J.& Neves, E.G. 2006. Black carbon increases cation exchange capacity in soils. *Soil Science Society of America Journal* **70**, 1719–1730.

Major, J., Rondon, M., Molina, D., Riha, S.J. & Lehmann, J. 2012. Nutrient leaching in a Colombian savanna Oxisol amended with biochar. *J. Environ. Qual*.**41**, 1076–1086.

Matieu, C. & Pielain, F. 2003. *Soil chemical analyses, selected methods (Analyses chimiques des sols, méthodes choisies).* Lavoisier, Paris, 387 pp.

Matsubara, Y., Hasegawa, N. & Fukui, H. 2002. Incidence of Fusarium Root Rot in Asparagus Seedlings Infected with Arbuscular Mycorrhizal Fungus as Affected by Several Soil Amendments. *Engei Gakkai zasshi*. **71**, 370–374.

Nelson, N.O., Agudelo, S.C., Yuan, W. & Gan, J. 2011. Nitrogen and Phosphorus Availability in Biochar-Amended Soils.*Soil Science* **176**(5), 218–226. doi:http://dx.doi.org/10.1097/SS.0b013e3182171eac

Noguera, D., Rondon, M., Laossi, K.R., Hoyos, V., Lavelle, P., Cruz de Carvalho, M.H. & Barotf, S. 2010. Contrasted effect of biochar and earthworms on rice growth and resource allocation in diffrent soils. *Soil Biology & Biochemistry* **42**, 1017–1027.

Novak, J.M., Busscher, W.J., Watts, D.W., Amonette, J.E., Ippolito, J.A., Lima, I.M., Gaskin, J., Das, K.C., Steiner, C., Ahmedna, M., Rehran, D. & Schomberg, H. 2012. Biochars impact on soil moisture storage in an Ultisol and two Aridisols. *Soil Sci*. **177**, 310–320.

Olsen, S.R., Cole, C.V., Watanabe, W.S & Dean, L.A. 1954. *Estimation of available phosphorus in soil by extrait with sodium bicarbonate*. USDA, Washington, 37 pp.

Sanchez-Garcia, M., Alburquerque, J.A., Sanchez-Monedero, M.A., Roig, A. & Cayuela, M.L. 2015. Biochar accelerates organic matter degradation an denhances N mineralisation during composting of poultry manure without a relev antimpacton gas emissions. Bioresour. *Technol*. **192**, 272–279.

Sohi, S. 2012. Carbon storage with benefits. *Science* **338**, 1034–1035.

Spokas, K.A. & Reicosky, D.C. 2009. Impacts of sixteen different biochars on soil greenhouse gas production. *Annals of Environmental Science* **3**, 179–193.

Spokas, K.A., Cantrell, K.B., Novak, J.M., Archer, D.W., Ippolito, J.A., Collins, H.P., Boateng, A.A., Lima, I.M., Lamb, M.C., McAloon, A.J., Lentz, R.D. & Nichols, K.A. 2012. Biochar : a synthesis of its agronomic impact beyond carbon sequestration. *J.Environ Qual*. **41**, 973–989.

Steinbeiss, S, Gleixner, G. & Antonietti, M. 2009. Effect of biochar amendment on soil carbon balance and soil microbial activity. *Soil Biology and Biochemistry* **41**, 1301–1310.

Steiner, C., Das, K.S., Melear, N. & Lakly, D. 2010. Reducing nitrogen loss during poultry litter composting using biochar. *J. Environ. Qual*. **39**(4), 1236–1242.

Steiner, C., Teixeira, W.G., Lehmann, J., Nehls, T., De Macedo, J.L.V., Blum, W.E.H. & Zech, W. 2007. Long term effects of manure, charcoal and mineral fertilization on cropproduction and fertility on a highly weathered Central Amazonian upland soil. *Plant Soil*. **291**, 275–290.

Tammeorga, P., Simojokib, A., Mäkeläa, P., Stoddarda, F.L., Alakukkuc, L & Helenius, J. 2014. Short-term effects of biochar on soil properties and wheat yieldformation with meat bone meal and inorganic fertiliser on a borealloamy sand. *Agriculture, Ecosystems and Environment* **191**, 108–116.

Xu, G., Sun, J., Shao, H. & Chang, S.X. 2014. Biochar had effects on phosphorus sorption and desorption in three soils with differing acidity. *Ecol. Eng*. **62**, 54–60.

Zhang, W., Niu, J., Morales, V.L., Chen, X., Hay, A.G., Lehmann, J. & Steenhuis, T.S. 2010. Transport and retention of biochar particles in porous media: effect of pH, ionic strength, and particle size. *Ecohydrology* **3**, 497–508.

Zhang, J., Lü, F., Shao, L. & He, P. 2014. The use of biochar-amended composting to improve the humification and degradation of sewage sludge. Bioresour. *Technol*. **168**, 252–258.

Zheng, H.2013. Impacts of adding biochar on nitrogen retention and bioavailability in agricultural soil. *Geoderma* **206**, 32–39.

Agronomy Research **17**(6), 2386–2398, 2019
https://doi.org/10.15159/AR.19.198

Pre-emergence grass weed control in winter wheat (*Triticum aestivum* L.) with soil applied premixed herbicides influenced by precipitations

Z. Pacanoski[1] and A. Mehmeti[2,*]

[1]Ss. Cyril and Methodius University in Skopje, Faculty of Agricultural Sciences and Food, 16-ta Makedonska brigada 3, MK1000 Skopje, Republic of North Macedonia
[2]University of Prishtina, Department of Plant Protection, Bill Clinton p.n., XK10000 Prishtina, Republic of Kosovo
[*]Corresponding address: arben.mehmeti@uni-pr.edu

Abstract. The field trials were conducted during two winter wheat growing seasons (2013–2014 and 2014–2015, respectively) to estimate weed control, and influence of herbicides on grain yield with PRE premixes in winter wheat crops. The field trials were conducted with 'Ingenio' and 'Pobeda' winter wheat cultivars which were sowed in a well-prepared soil seedbed at a seeding rate of 220 and 240 kg ha^{-1}. The experimental design was a randomised complete block with four replicates and elementary plots 25 m^2. The efficacy of PRE herbicides varied with treatments among weed species and periods of efficacy estimation, regions and years, respectively. Overall, the performance of the PRE herbicide premixes correlated with the weather conditions. All PRE herbicide premixes effectively reduced the dominant weed species *Milium vernale*, *Papaver rhoeas,* and *Galium aparine* in the Bitola region in 2013–2014, but not in 2014–2015 due to heavy rain during the first two weeks of herbicide application. In contrast, the limited precipitation after PRE application may have contributed to the poor performance of PRE herbicides in the Probištip region in 2013 compared with 2014. In the Bitola region, the lowest crop yield was obtained in plots treated with diflufenican + isoproturon (2,960 kg ha^{-1}) in both growing seasons. In the Probištip region, the wheat grain yields in 2013–2014 following all PRE applied herbicides were significantly lower (between 520 and 800 kg ha^{-1}) than weed-free control. In 2014–2015, diflufenican + isoproturon herbicide treatment produced the lowest yield of 2,530 kg ha^{-1}, whereas chlortoluron + triasulfuronwas the highest-yielding herbicide treatment (2,820 kg ha^{-1}). However, results indicated that in Bitola region comparatively higher yield were found in plots treated with chlortoluron + triasulfuron (3,450 kg ha^{-1}), in both growing seasons, also in Probistip region herbicide chlortoluron + triasulfuron achieved higher yield (2,820 kg ha^{-1}), in both growing seasons).

Key words: PRE herbicide, efficacy, weeds, wheat.

INTRODUCTION

Winter wheat is important crop grown on approximately 15% of the arable land in Republic of Macedonia (Anonymous, 2017). It is a popular rotational crop helping to maintain soil structure and break weed cycles, as weeds comprise the most undesirable, aggressive and troublesome element of crop production (Kazi et al., 2007). Among the factors which adversely affect wheat crop yields, weed infestation is the most harmful, but less noticeable (Oad et al., 2007). Wheat often suffers from competition from numerous weed species, where the reduction of wheat yields due to weed infestation can reach on average up to 23% globally (Oerke, 2006).

For this reason, one of the most significant aspects of winter wheat production is weed management. Substantial crop yield and associated economic losses can occur if weeds are not adequately controlled. Under certain conditions, weeds have their greatest detrimental effect on the ultimate yield of winter wheat during the early stages of the crop development (Pilipavičius, 2012), so the pre-emergence herbicides can play a valuable role in controlling weeds at these critical growth stages (Shehzad et al., 2012; Kazmarek & Matysiak, 2015; Kaur et al., 2018). Pre-emergent herbicides offer an alternate mode of action to many post-emergent options, reducing selection pressure on subsequent post-emergent herbicide applications and removing much of the early season weed competitive pressure on crops by controlling weeds that emerge with winter wheat.

Herbicides registered for PRE weed control in winter wheat in the Republic of Macedonia have significantly changed over the past 4–5 years. 'Forgotten' and 'old' well-known herbicide active ingredients are in favour once again. Among them, different combinations of pendimethalin, isoproturon, chlortoluron and isoxaben are the most frequently applied. Soil residual activity may be maintained for 16 (chlortoluron) (Tomlin, 1994) to 20–25 weeks (pendimethalin, izoxaben and isoproturon) (Vouzounis & Americanos, 1995; Alletto et al., 2006) insuring long lasting weed control over the prolonged period of weed emergence.

The main objectives of the research were (i) to determine whether acceptable weed control of PRE premixes in winter wheat crop may replace usually spring POST-applied herbicides, and (ii) to evaluate their injury effect and influence on the winter wheat yield.

MATERIALS AND METHODS

The field trials were conducted during two winter wheat growing seasons in 2013–2014 and 2014–2015 on commercial wheat fields in the Bitola and Probištip wheat growing regions in south-western and north-eastern Macedonia on molic-vertic gleysol and vertisol, respectively (Filipovski, 2006) (Table 1).

Table 1. Soil characteristics in the wheat-growing regions

Region	Soil	coarse, %	fine sand, %	clay+silt, %	organic matter, %	pH-water, %
Bitola	molic-vertic gelysol	27.10	47.30	25.60	1.86	6.30
Probištip	Vertisol	3.50	30.00	60.30	2.40	7.20

The wheat was grown following conventional tillage practices. The soil was tilled with a field cultivator prior to sowing. Nitrogen, phosphorus and potassium were applied as per soil test-based recommendation. The field trials were carried out with 'Ingenio' and 'Pobeda' winter wheat cultivars sowed in a well-prepared soil at a seeding rate of 220 and 240 kg ha^{-1} on 10th October 2013 and 18th October 2014 in the Bitola region, and on 12th October 2013 and 21st October 2014 in the Probištip region, respectively. The trials were conducted in two different sites of the same commercial wheat fields. Herbicides were applied with a CO_2-pressurised backpack sprayer calibrated to deliver

300 L ha^{-1} solution at 220 kPa. Herbicides were applied to the dry seed, at the beginning of the seed imbibitions wheat growing stage (BBCH 00-01). Weeds at the time of treatment were in the same growth stages as wheat (BBCH 00-01). The experimental design was a randomised complete block with four replicates and elementary plots 25 m^2, in total were six treatments (Table 2).

Table 2. Basic data for applied herbicides

Treatments	Trade name	Rate	Time of application
chlortoluron 500 g L^{-1} + isoxaben 18.7 g L^{-1}	Aubaine	3.6 L ha^{-1}	pre-emergence
pendimethalin 250 g L^{-1} + isoproturon 125 g L^{-1}	Maraton 375 SC	4.0 L ha^{-1}	pre-emergence
chlortoluron 500 g L^{-1} + triasulfuron 750 g kg^{-1}	Tolurex 50 SC Logran 75 WG	3.2 L ha^{-1} 0.037 kg ha^{-1}	pre-emergence
diflufenican 50 g L^{-1} + isoproturon 500 g L^{-1}	Quartz Super	1.6 L ha^{-1}	pre-emergence
Weed free control			
Control (untreated)			

The weed control plots were left untreated during the entire experimental period. Weed-free control was maintained by hand weeding, which was initiated at weed emergence and continued as required to maintain weed-free plots.

Weed control efficacy was estimated 28 days after application (DAA) (the first estimation) and in the spring, 150 DAA at the middle of wheat tillering stage (BBCH 24-26) (the second estimation) by counting the weed plants in a 1 m^2area within each plot. Herbicide efficacy was calculated by equation according to Hasanuzzaman et al. (2008):

$$\mathrm{Wce} = \frac{\mathrm{Wup} - \mathrm{Wtp}}{\mathrm{Wup}} \cdot 100 \qquad (1)$$

where W_{ce} – weed control efficiency; Wup – number of weeds in the untreated plots; Wtp – number of weeds in the treated plots.

Wheat injury was visually evaluated based on a 0–100% rating scale, where 0 is no injury to wheat plants and 100 is complete death of wheat plants (Frans et al., 1986). Visual estimates of the percentage wheat injury were estimated 7 and 21 days after emergence (DAE), based on chlorosis and necrosis for each plot at both localities during the two-year experimental period. At full maturity, wheat was harvested manually at ground level in an area of 1 m² per plot and the yield was determined after harvest based on weights of grain containing 13% moisture.

Total weekly rainfall, as well as weekly average temperatures 1 week before and 4 weeks after PRE applications were recorded, respectively (Table 3). Precipitation 1 week before and 4 weeks after PRE applications in 2013 were in line with the average for the Bitola region, but scarce for the Probištip region. In the Bitola region, precipitation occurred on the 2nd day of the week before PRE applications, and on the first 2 and the last day of the 1st and the last 2 days of the 3rd week after PRE applications. In contrast, in the Probištip region in the same year, it rained on 7 and 11 days at intervals throughout the 1- and 4-week period before and after PRE applications. Furthermore, the 4-week period after PRE application in autumn of 2014 in Bitola region was unusually wet, particularly the 2nd, 3rd, and 4th day of the 1st week, as well as 3 days in the middle of the 2nd and the last 2 days of 4th week. This period was also very humid, 56% above the 30-year average forthe Bitola region (106 mm). There was no precipitation 1 week before PRE application, however, precipitation occurred in the 1st, 2nd, and 4th week after PRE application, as well as in the 1st week before PRE applications in the Probištip region for the same year, in the line with the average for this region (11, 13, 8, and 14 mm). The 3rd week was wetter in comparison with previous weeks (23 mm) (Table 3).

The temperature 1 week before and 4 weeks after PRE application for both years and regions was slightly above the average and that was attributed to favourable environmental conditions associated with no night frosts during the estimated 1- and 4-week period before and after PRE application. PRE treatments in both years were applied at times when herbicide applications typically occur in Macedonia wheat production, thus are representative of producer practices and label recommendations.

The data were tested for homogeneity of variance and normality of distribution (Ramsey & Schafer, 1997) and were log-transformed as required to obtain equal variances and better symmetry before ANOVA was performed. Data were transformed back to their original scale for presentation. Means were separated using the LSD test at 5% probability.

Table 3. Mean weekly temperatures and total weekly rainfall 1 week before application (WBA) and 4 weeks after application (WAA) PRE applications, respectively at Bitola and Probištip region in 2013 and 2014

	Bitola region				Probištip region			
	2013		2014		2013		2014	
Weeks	P (mm)	T (ºC)	P (mm)	T (ºC)	P (mm)	T (ºC)	P (mm)	T (ºC)
1st WBA	8	13	0	15	5	15	11	17
1st WAA	14	9	37	10	7	10	13	14
2nd WAA	10	11	31	7	2	14	8	11
3rd WAA	21	8	11	8	9	9	23	12
4th WAA	6	6	27	7	13	9	14	9

RESULTS AND DISCUSSION

Weed control. Efficacy of PRE herbicides varied among weed species, treatments, periods of efficacy estimation, regions and years. Overall, the performance of the PRE herbicide premixes correlated with the weather conditions. Both sites were naturally infested with a high population of *Milium vernale* M. Bieb., *Papaver rhoeas* L., *Galium aparine* L. *Avena ludoviciana* Dur., *Alopecurus myosuroides* Huds., *Bifora radians* M.B. and *Polygonum convolvulus* L. Inconsistent weather patterns between the two study growing seasons probably influenced weed control. The humid autumn in 2014 (Table 3), particularly heavy rain (68 mm) during the 1st and 2nd week of herbicide applications and before weed emergence, may have promoted leaching of herbicides from the soil surface, resulting in the lower efficacy of PRE applied premixes in 2014 compared to 2013 in the Bitola region (Table 3). Weed density was higher due to the climatic condition in the non-treated control plot was 93 and 135 plants m^{-2} in 2013–2014 and 2014–2015, respectively in the Bitola region, and 85 and 104 plants m^{-2} in 2013–2014 and 2014–2015 respectively in the Probištip region. In contrast, the limited precipitation after PRE application may have contributed to the poor performance of PRE herbicides in the Probištip region in 2013 compared with 2014 (Table 3).

***Milium vernale*.** The control of *M.vernale* in Bitola region was significant among years with PRE herbicides, but *M. vernale* control did not differ between periods of efficacy estimation by year. In this region in 2013–2014, 28 DAA, all PRE applied premixes controlled *M. vernale* between 87 and 100%, whereas excellent control (> 95%) was achieved withchlortoluron + isoxaben and chlortoluron + triasulfuron. Negligible lower *M. vernale* control was obtained 150 DAA with all herbicide treatments in 2014–15. The efficacy of all PRE applied premixes ranged between 82% and 96%, probably due to the residual activity of PRE herbicides and no new spring growth of *M. vernale*, a typical winter weed (Kostov, 2006). In contrast, all PRE herbicide premixes provided poor control of *M. vernale* in 2014–2015, regardless of the period of efficacy estimation, the herbicides controlled *M. vernale* between 58 and 78% (Table 4). The differences in the control of *M. vernale* between the years indicated that higher amounts of precipitation (37 mm) after herbicide application in 2014–2015 caused herbicides to leach through the soil profile, consequently decreasing weed control efficacy (Ferrell et al., 2004; Tanji & Boutfirass, 2018).

***Papaver rhoeas*.** The control of *P. rhoeas* in Bitola region was significant among years with PRE herbicides, while *P. rhoeas* control did not differ between the periods of efficacy estimation by year. A significant treatment by year interaction resulted in two distinct years for *P. rhoeas* control at Bitola region with PRE herbicide premixes. but *P. rhoeas* control did not differ among periods of efficacy estimation by year. PRE herbicide premixes controlled *P. rhoeas* more than 98% at 28 DAA in 2013–2014 and with similar efficacy at 150 DAA. PRE applied premixes in 2014–2015 did not achieve more than 74% (28 DAA) and 70% (150 DAA) control of *P. rhoeas,* respectively (Table 4). PRE herbicides require precipitation to move into the zone of active weed seed germination (Chomas & Kells, 2004), so heavy precipitation immediately after PRE applications in 2014 and leaching of herbicides out of the weed seed germination zone caused significant variability among PRE treatments in the control of *P. rhoeas* in both years. The efficacy of PRE applied mixtures pendimethalin + linuron, beflubutamid + isoproturon, and pyraflufen + isoproturon in the control of *P. rhoeas* in winter cereals ranged from 93 to 100% (Torra et al., 2010). These values are within the efficacy range for *P. rhoeas* control reported by Cirujeda et al. (2000) and Garcia de Arevalo et al. (1992) reported that similar mixtures of PRE herbicides controlled *P. rhoeas* in field beans.

***Galium aparine*.** A significant treatment by year interaction resulted in two distinct years for *G. aparine* control in Bitola region with PRE herbicide premixes. *G. aparine* control did not differ between the periods of efficacy estimation by year. In 2013–2014, all investigated PRE premixes provided more than 95% control of *G. aparine* for both periods of efficacy estimation. However, the control drastically decreased the following year, probably due to the Bitola region receiving 68 mm precipitation in the 1st and 2nd week of herbicide applications. It is likely that these extremely humid conditions contributed to the decrease in efficacy of PRE herbicides, which ranged between 57% and 67% (Table 4). Isoproturon tank-mixed with pendimethalin provided the most satisfactory residual control of *G. aparine* in winter wheat (Gianessiet al., 2003). Lovegrove et al. (1985) assessed the efficacy of a range of PRE herbicides for the control of *G. aparine*, reporting that pre-emergence applications of pendimethalin, trifluralin with linuron and bifenox with linuron gave inadequate control.

Avena ludoviciana. *Avena ludoviciana* control did not differ among regions, years, and periods of efficacy estimation for PRE herbicide premixes. PRE herbicides, regardless of the growing season,had little effect on *A. ludoviciana* in both regions. In the Bitola region, control of *A. ludoviciana* was less than 69% and 63% with any PRE treatment 28 DAA and 150 DAA, respectively in 2013–2014, with similar efficacy (< 63% and < 58%, respectively) recorded in 2014–2015 (Table 4). In the Probištip region, PRE herbicide premixes provided no more than 49% and 67% control of *A. ludoviciana* in 2013–2014 and 2014–2015, respectively (Table 5). In general, pre-emergence herbicides do not effectively control *A. ludoviciana* (Thomas & Yaduraju 2000), because the seeds of *A. ludoviciana* have the ability to germinate from greater depths in the soil (under certain conditions from depths as great as 25 cm) (Kostov, 2006). Dormancy and multiple emerging flushes throughout the growing season results in the persistence and continual re-infestation of this weed in the soil seed bank (Fuerst et al., 2011; Beckie et al., 2012). Poor wild oat control and, at the same time, the highest dry weights of this weed (9.43, 8.70 and 8.16 g m^{-2}), were recorded in plots treated with isoproturon at 1.5 a.i kg ha^{-1}, isoproturon + diflufenicon at 0.98 a.i. kg ha^{-1}, isoproturon + carfentrazone ethyl at 2.0 a.i. kg ha^{-1}, respectively (Shehzad et al., 2012). In contrast, Singh and Gosh (1992) reported that the application of pendimethalin and isoproturon before emergence provided maximal *A. ludoviciana* control.

Alopecurus myosuroides. A significant treatment by year interaction resulted in two distinct years for *A. myosuroides* control in Probištip region with PRE herbicides. However, the control of *A. myosuroides* did not differ among periods of efficacy estimation by year. In 2013–2014, PRE herbicides provided lower efficacy for control of *A. myosuroides* 52% and 61% (28 DAA) and 48% and 59% (150 DAA).The lack of effective control of *A. myosuroides* can be attributed to the low rainfall after PRE application in 2013 (Table 3). Since many of the PRE herbicides can volatilise and photodegrade on the soil surface over time, rainfall is need to transfer these herbicides into the zone where weed seeds germinate (Janak & Grichar, 2016), which explains the inconsistent control of *A. myosuroides* observed with PRE herbicide premixes under the drought conditions in the Probištip region in autumn 2014. Control of *A. myosuroides* improved in 2014–2015, due to the sufficient precipitation (11 mm 1^{st} WBA and 13 mm 1^{st} WAA, respectively) increasing the efficacy of the PRE herbicide premixes; 28 DAA, all PRE applied premixes controlled *A. myosuroides* between 92 and 100%. The high efficacy in *A. myosuroides* control was maintained 150 DAA, as *A. myosuroides* shares the same growth cycle as autumn-sowed wheat (Orson & Thomas, 2001), which means it is typical winter weed (Kostov, 2006) and no new spring growth was recorded. Diflufenican + isoproturon provided at least 87% control of *A. myosuroides*, while pendimethalin + isoproturon, chlortoluron + isoxaben, and chlortoluron + triasulfuron provided better control than diflufenican + isoproturon (95–100%) (Table 5). Single applications of isoproturon or chlorotoluron in the UK have been reported to effectively control *A. myosuroides* (Orson & Harris, 1997). According Moss et al. (2009), PRE flufenacet + pendimethalin (240 + 1,200 g a.i. ha) achieved 93% reduction of *A. myosuroides*, while PRE flufenacet + pendimethalin followed by isoproturon + pendimethalin (1,500 + 1,320 g a.i. ha) achieved 97% reduction.

Table 4. *Milium vernale,Papaver rhoeas, Galium aparine* and *Avena ludoviciana* control (%) 28 and 150 days after PRE herbicide applications, in winter wheat in 2013–2014 and 2014–2015 in Bitola region

Treatments	Bitola region															
	MIVER				GALAP				PAPRH				AVELU			
	2013–2014		2014–2015		2013–2014		2014–2015		2013–2014		2014–2015		2013–2014		2014–2015	
	28 DAA	150 DAA	28 DAA	150 DAA	28 DAA	150 DAA	28 DAA	150 DAA	28 DAA	150 DAA	28 DAA	150 DAA	28 DAA	150 DAA	28 DAA	150 DAA
Weedy control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
chlortoluron+ isoxaben	95^{a}	92^{a}	74^{ab}	66^{ab}	100^{a}	100^{a}	66^{a}	62^{a}	100^{a}	100^{a}	74^{a}	70^{a}	58^{b}	55^{a}	63^{a}	58^{a}
pendimethalin+ isoproturon	87^{b}	83^{b}	68^{bc}	61^{bc}	95^{b}	100^{a}	63^{ab}	57^{ab}	98^{a}	100^{a}	68^{ab}	66^{ab}	54^{b}	57^{a}	59^{ab}	53^{a}
chlortoluron + triasulfuron	100^{a}	96^{a}	78^{a}	69^{a}	100^{a}	100^{a}	67^{a}	64^{a}	100^{a}	100^{a}	69^{ab}	64^{b}	67^{a}	63^{a}	55^{b}	52^{a}
diflufenican + isoproturon	89^{b}	82^{b}	65^{c}	56^{c}	97^{ab}	98^{a}	59^{b}	50^{b}	100^{a}	100^{a}	65^{b}	63^{b}	69^{a}	59^{a}	63^{a}	54^{a}
LSD 0.05	5.27	5.76	8.36	6.35	3.05	2.00	6.15	7.35	2.45	1.01	8.03	5.46	5.89	9.08	6.09	8.81
Random effect interactions PRE herbicides treatment x year	*				*				*				NS			
PRE herbicides treatment x PEE	NS		*		NS			NS	NS			NS	NS			NS

[a]Abbreviation: PRE – preemergence; DAA – days after application; MIVER – *Milium vernale*; PAPRH – *Papaver rhoeas*; AVELU – *Avena ludoviciana*; GALAP – *Galium aparine*; PEE – periods of efficacy estimation; NS – not significant; * Significant at the 5% level according to a Fisher's protected LSD testat $p < 0.05$.

PRE treatments were applied in the same growth stages as wheat (beginning of seed imbibitions wheat growing stage – BBCH 00-01).

Weed control efficacy was estimated 28 DAA and 180 DAA.

Means followed by the same letter within a column are not significantly different according to Fisher's Protected LSD at $p < 0.05$.

Table 5. *Avena ludoviciana*, *Alopecurus myosuroides*, *Bifora radians* and *Polygonum convolvulus* control (%) 28 and 150 days after PRE herbicide applications, in winter wheat in 2013–2014 and 2014–2015 in Probištip region

Treatments	Probištip region															
	AVELU				ALOMY				BIFRA				POLCO			
	2013–2014		2014–2015		2013–2014		2014–2015		2013–2014		2014–2015		2013–2014		2014–2015	
	28 DAA	150 DAA	28 DAA	150 DAA	28 DAA	150 DAA	28 DAA	150 DAA	28 DAA	150 DAA	28 DAA	150 DAA	28 DAA	150 DAA	28 DAA	150 DAA
Weedy control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
chlortoluron + isoxaben	44^{a}	47^{a}	61^{ab}	63^{a}	57^{ab}	59^{a}	98^{a}	96^{a}	-	52^{ab}	-	58^{a}	-	49^{ab}	-	55^{ab}
pendimethalin + isoproturon	49^{a}	41^{a}	59^{ab}	64^{a}	53^{ab}	49^{b}	95^{ab}	95^{a}	-	48^{bc}	-	54^{a}	-	51^{a}	-	57^{a}
chlortoluron + triasulfuron	37^{b}	43^{a}	67^{a}	62^{a}	61^{a}	57^{a}	100^{a}	100^{a}	-	54^{a}	-	57^{a}	-	50^{a}	-	52^{ab}
diflufenican + isoproturon	28^{c}	25^{b}	55^{b}	53^{b}	52^{b}	48^{b}	92^{b}	87^{b}	-	45^{c}	-	52^{a}	-	42^{b}	-	48^{b}
LSD 0.05	6.52	7.19	8.56	7.72	8.05	7.15	4.40	5.29	-	5.67	-	7.38	-	7.58	-	8.09
Random effect interactions PRE herbicides treatment x year	NS				*				NS				NS			
PRE herbicides treatment x PEE	NS		NS		NS		NS		-		-		-			-

Abbreviation: PRE – preemergence; DAA – days after application; AVELU – *Avena ludoviciana*; ALOMY – *Alopecurus myosuroides*; BIFRA – *Bifora radians*; POLCO – *Polygonum convolvulus*; PEE – periods of efficacy estimation; NS – not significant; * Significant at the 5% level according to a Fisher's protected LSD testat $p < 0.05$.
PRE treatments were applied in the same growth stages as wheat (at dry seed – beginning of seed imbibitionswheat growing stage – BBCH 00–01).
Weed control efficacy was estimated 28 DAA and 180 DAA.
Means followed by the same letter within a column are not significantly different according to Fisher's Protected LSD at $p < 0.05$.

Table 6. Wheat plant injury as influenced by PRE applied herbicide premixes, and grain yield as influenced by PREapplied herbicide premixes in winter wheat in Bitola and Probištip region in 2013–2014 and 2014–2015[a–d]

Treatments	Rate (g L ha^{-1})	Bitola region						Probištip region					
		Wheat injury				Grain yield (kg ha^{-1})		Wheat injury				Grain yield (kg ha^{-1})	
		2013–2014		2014–2015				2013–2014		2014–2015			
		7 DAE	21 DAE	7 DAE	21 DAE	2013–2014	2014–2015	7 DAE	21 DAE	7 DAE	21 DAE	2013–2014	2014–2015
Weedy control	------	0	0	0	0	2,180^{e}	2,060^{d}	0	0	0	0	1,760^{d}	2,040^{d}
Weed-free control	------	0	0	0	0	3,910^{a}	3,940^{a}	0	0	0	0	3,110^{a}	3,570^{a}
chlortoluron+ isoxaben	3.6	0	0	7	2	3,380^{bc}	2,880^{b}	0	0	0	0	2,430^{bc}	2,780^{b}
pendimethalin + isoproturon	4.0	0	0	20	16	3,230^{c}	2,750^{c}	0	0	0	0	2,370^{bc}	2,580^{c}
chlortoluron + triasulfuron	3.2 + 0.037	0	0	9	3	3,450^{b}	2,960^{b}	0	0	0	0	2,590^{b}	2,820^{b}
diflufenican + isoproturon	1.6	0	0	22	17	2,960^{d}	2,700^{c}	0	0	0	0	2,310^{c}	2,530^{c}
LSD 0.05						170.76	118.57					221.29	148.25
Random effect interactions PRE herbicides x year		*				*		NS				*	

Abbreviation: PRE – preemergence; DAA – days after application;NS – not significant; * Significant at the 5% level according to a Fisher's protected LSD testat $p < 0.05$.
PRE treatments were applied in the same growth stages as wheat (at dry seed – beginning of seed imbibitionswheat growing stage – BBCH 00-01).
Wheat injury was estimated 7 and 21 days after emergence (DAE).
Means followed by the same letter within a column are not significantly different according to Fisher's Protected LSD at $p < 0.05$.

Bifora radians and ***Polygonum convolvulus*.** Taking into consideration that *B. radians* and *P. convolvulus* are annual broadleaf early-germinating spring weeds (Mennan & Zandstra, 2005; Altop & Mennan, 2017; Majd et al., 2016), no data are presented regarding the efficacy of PRE herbicide premixes 28 DAA for both years in the Probištip region. Soil-applied herbicides controlled *B. radians* and *P. convolvulus* 150 DAA due to their soil residual activity. However, the efficacy of PRE herbicide premixes was poor and did not differ among years, providing no more than 54% and 63% control of *B. radians* in 2013–2014 and 2014–2015, respectively. Identical efficacy was recorded for *P. convolvulus* control for both years (51% and 62%, respectively) (Table 5). Similarly, Durbin (2017) reported that PRE herbicides applied in late fall/winter cannot be fully effective because seeds of *P. convolvulus* germinate in early spring. Marković et al. (2005) also reported that chlorotoluron is insufficiently effective on many weeds, including *Bifora radians.*

Wheat injury. Higher precipitation (68 mm) in autumn 2014 directly following PRE herbicide treatments caused wheat injury in the Bitola region, ranging from 7% to 22% across PRE treatments 7 days after emergence (DAE). However, injures as a result of premixes containing diflufenican + isoproturon and pendimethalin + isoproturon were more serious (22 and 20%, respectively).

Furthermore, in 2014–2015 in Bitola region, heavy precipitation occurred in the 1st and 2nd week of herbicide applications, causing leaching of herbicides through the soil profile. Injuries caused by chlortoluron + isoxaben and chlortoluron + triasulfuron (premixes that contain chlortoluron) significantly decreased by 7 and 21 DAA (Table 6). However, wheat injuries of diflufenican + isoproturon and pendimethalin + isoproturon were still evident at 21 DAE. By the time, wheat compensated for early injuries in its growth, the crop fully recovered and did not suffer any yield reduction. PRE applied chlorotoluron (2,000 g ha^{-1}) + isoxaben (74.8 g ha^{-1}), prosulfocarb (4,000 g ha^{-1}), prosulfocarb (2,000 g ha^{-1}) + s-metolachlor (300 g ha^{-1}), and pendimethalin (1,320 g ha^{-1}) reduced wheat emergence and density because of heavy rain (about 100 mm) after herbicide treatments and before crop emergence (Tanji & Boutfirass, 2018).

Wheat grain yield. Wheat grain yields for each treatment in both regions generally reflected overall weed control (Table 6). Comparison of weedy and weed-free control indicated that weeds reduced wheat grain yield by 44 and 48% in the Bitola region, and 43% in the Probištip region for both years (Table 6). Similar, Kaur et al. (2018) noted that the season long growth of weeds reduced the wheat yield up to 38.5%. A significant treatment by year interaction resulted in two distinct years for wheat grain yield in the Bitola region. In both years, the lowest wheat grain yield was recorded in untreated control plots (2,180 and 2,060 kg ha^{-1}, respectively). The lowest yield between herbicide premixes in 2013–2014 was obtained in plots treated with diflufenican + isoproturon (2,960 kg ha^{-1}).

The yield was no higher than the weed-free control with any PRE applied herbicide in both growing seasons. Wheat yields were more closely related to the percentage weed control than wheat injury, with only the weed-free control producing a statistically higher wheat yield compared to all evaluated herbicides (Table 6). A significant treatment by year interaction also resulted in two distinct years for wheat yields in the Probištip region with PRE herbicides. In 2013–2014, wheat grain yields following all PRE applied herbicides were significantly lower (between-520 and -800 kg ha^{-1}) than weed-free control (Table 6), whereas in 2014–2015, wheat yields ranged from 2,040 to

3,570 kg ha^{-1}. Diflufenican + isoproturon was the lowest-yielding herbicide treatment with 2,530 kg ha^{-1}, whereas chlortoluron + triasulfuron was the highest-yielding herbicide treatment (2,820 kg ha^{-1}) (Table 6).

The increases in grain yield resulting from isoproturon+ diflufenican application amounted to 54.9% in the first season and 57.9% in the second season over the unweeded (El Metwally et al., 2015).

Moreover, Kieloch & Rola (2010) reported that impact of the mixture of pendimethalin + isoproturon on grain yield of Clever cultivar was observed only in the season with hard winter conditions. Application of pendimethalin 1.0 kg ha^{-1}or metribuzin 0.21 kg ha^{-1} or a tank-mix of pendimethalin 0.75 and 1.0 kg ha^{-1} + metribuzin at 0.175 kg ha^{-1} resulted in statistically similar wheat grain yields (4.68 and 4.74 t ha^{-1}) to two hand weeding (4.8 t ha^{-1}) (Kaur et al., 2018). Significantly, a negative linear relationship between winter wheat yield and the number of *Lolium rigidum* was reported in study by Tanji & Boutfirass (2018). According to their results, chlortoluron + isoxaben provided 93% control of *Lolium rigidum*and was the highest-yielding herbicide treatment with 9.8, 6.8 and 8.9 t ha^{-1} in three separate experiments.

CONCLUSIONS

These results demonstrated that the efficacy of PRE herbicides in wheat crops are strongly dependent on the amount of precipitation and weed populations. The humid autumn in second year of study and particularly heavy rain after two weeks of herbicide applications probably resulted in leaching of herbicides from soil surface in Bitola region. Futhermore, for that efficay of herbicides were lower in 2014 compared to 2013 in Bitola region. Consequently, chlortoluron + isoxaben, pendimethalin + isoproturon, chlortoluron + triasulfuron, and diflufenican + isoproturon effectively reduced the dominant *Milium vernale*,*Papaver rhoeas*, and *Galium aparine* in 2013–2014, but not in 2014–2015. In contrast, the limited precipitation 7 days before and after herbicide application in the Probištip region in 2013 reduced the efficacy of PRE herbicides that require precipitation for optimal activity and weed control. Dry or very humid environmental and soil conditions immediately after PRE autumn herbicide application were accompanied by poor control of the deeper germinating *A. ludoviciana*, as well as poor control of the spring germinating *B. radians* and *P. convolvulus*, indicating that any combination of PRE herbicides must be followed by POST herbicides for control of escaped and newly emerging weeds. Therefore, the amount of precipitation and weed flora should be considered when selecting the most appropriate PRE weed management strategy for winter wheat crops. Based on the results presented, we recommend the usage of traisulfuron+dicamba or tribenuron-methyl in the study region for successful weed control and high wheat grain yields.

REFERENCES

Alletto, L., Coquet, Y., Benoit, P. & Bergheaud, V. 2006. Effects of temperature and water content on degradation of isoproturon in three soil profiles. *Chemosphere* **64**(7), 1053–1061. doi: 10.1016/j.chemosphere.2005.12.004

Altop, E.K. & Mennan, H. 2017. Determination of morphological and biological diversity of acetolactate synthase inhibitor herbicides resistant and susceptible wild bishop (*Bifora radians* M. Bieb.) populations. *Turkish Journal of Agriculture – Food Science and Technology* **5**(11), 1301–1306. doi: https://doi.org/10.24925/turjaf.v5i11.1301-1306.1349

Anonymous. 2017. Agricultural Statistic of Republic of Macedonia, Ministry for agriculture, forestry and water utilization, Government of R. of Macedonia, Skopje, R. of Macedonia.

Beckie, H.J., Francis, A. & Hall, L.M. 2012. The biology of Canadian weeds, 27: *Avena fatua* L (updated). *Canadian Journal of Plant Science* **92**(7), 1329–1357. doi: https://doi.org/10.4141/cjps2012-005

Chomas, A.J. & Kells, J.J. 2004. Triazine-resistant common lambsquarters (*Chenopodium album*) control in corn with preemergence herbicides. *Weed Technology* **18**(3), 551–554. doi: https://doi.org/10.1614/WT-03-077R

Cirujeda, A., Tarrago, R., Recasens, J. & Taberner, A. 2000. Herbicide control ofresistant *Papaver rhoeas* L. to tribenuron and 2,4-D. In: Proceedings of the 3rdInternational Weed Science Congress. International Weed Science Society, Fozdo Iguassu, Brazil, **15** pp. doi: https://doi.org/10.1017/S0021859605005708

Durbin, M. 2017. Weed identification and control. www.abctlc.com (866) 557–1746. Available on: http://www.abctlc.com/downloads/courses/WEEDS.pdf

El Metwally, I.M., Abd El- Salam, M.S. & Ali, O.A.M. 2015. Effect of zinc application and weed control on wheat yield and its associated weeds grown in zinc-deficient soil. *International Journal of Chem. Tech Research* **8**(4), 1588–1600.

Ferrell, M.A., Whitson, T.D. & Miller, S.D. 2004. Basic guide to weeds and herbicides. The University of Wyoming, College of Agriculture, Department of Plant Sciences, *Cooperative Extension Service*, *MP18*, pp. 1–19.

Filipovski, G. 2006. Soil classification of the Republic of Macedonia', MASA, pp. 313–323.

Frans, R.E., Talbert, R., Marx, D. & Crowley, H. 1986. Experimental design and techniques for measuring and analyzing plant responses to weed control practices. *In* N.D. Camper ed. Research Methods in Weed Science. 3rd ed. Champaign, IL: *Southern Weed Science Society*, pp. 37–38.

Fuerst, E.P., Anderson, J.V., Kennedy, A.C. & Gallagher, R.S. 2011. Induction of polyphenol oxidase activity in dormant wild oat (*Avena fatua*) seeds and caryopses: A defense response to seed decay fungi. *Weed Science* **59**(2), 137–144. doi: https://doi.org/10.1614/WS-D-10-00123.1

Garcia de Arevalo, R.C., Lusarreta, C.A., Neyra, C.B., Sanchez, M.A. & Algarra, P.J.H. 1992. Chemical control of annual weeds in field beans (*Vicia faba*) in central Spain. *Weed Science* **40**, 96–100.

Gianessi, L., Sankula, S. & Reigner, N. 2003. Plant Biotechnology: Potential impact for improving pest management in European agriculture wheat – Herbicide-Tolerant Case Study. The National Centre for Food and Agricultural Policy. Available on: http://www.ncfap.org/documents/ExecutiveSummaryJune.pdf.

Hasanuzzaman, M., Islam, M.O. & Bapari, M.S. 2008. Efficacy of different herbicides over manual weeding in controlling weeds in transplanted rice. *Australian Journal of Crop Science* **2**, 18–24.

Janak, T.W. & Grichar, W.J. 2016. Weed control in corn (*Zea mays* L.) as influenced by preemergence herbicides. *International Journal of Agronomy*, 1–9. doi: https://doi.org/10.1155/2016/2607671

Kaczmarek, S. & Matysiak, K. 2015. Application of reduced doses of chlorsulfuron in semi-dwarf and full-height cultivars of winter triticale. *Journal of Plant Protection Research* **55**(1), 8–15. doi: https://doi.org/10.1515/jppr-2015-0002

Kaur, E., Sharma, R. & Singh, N.D. 2018. Efficacy of pre-emergence and post emergence herbicides on weed control and yield in wheat. *International Journal of Current Microbiology and Applied Sciences* **7** (2), 883–887. doi: https://doi.org/10.20546/ijcmas.2018.702.111

Kazi, B.R., Buriro, A.H., Kubar, R.A. & Jagirani, A.W. 2007.Weed spectrum frequency and density in wheat, (*Triticum aestivum* L.) under Tandojam conditions. *Pakistan Journal of Weed Science Research* **13**(3–4), 241–246.

Kileoch, R. & Rola, H. 2010. Sensitivity of winter wheat cultivars to selected herbicides. *Journal of Plant Protection Research* **50**(1), 35–40. doi: https://doi.org/10.2478/v10045-010-0006-4

Kostov, T. 2006. *Herbology*. Scientific Book. The University Press of the Republic of Macedonia, Skopje, 371 pp.

Lovegrove, A.W., Lutman, P.J.W. & Thornton, M.E. 1985. Investigations into the control of cleavers (*Galium aparine*) with several pre- and post-emergence herbicides in winter cereals. *Aspects of Applied Biology*. **9**, 205–211.

Majd, B.A., Montazeri, M.M. & Younesabadi, M. 2016. The effect of different treatments on seed dormancy breaking and germination of *Polygonum convolvulus*. *International Journal of Farming and Allied Sciences* **5**(6), 427–433.

Marković, M., Protić, N., Protić, R. &Janković, S. 2005. New possibilities of weed control in wheat. *Romanian Agricultural Research* **22**, 41–46.

Mennan, H. & Zandstra, B.H. 2005. Influence of wheat seeding rate and cultivars on competitive ability of Bifra(*Bifora radians*). *Weed Technology* **19**, 128–136.

Moss, S.R., Hull, R. & Marshall, R. 2009. Control of *Alopecurus myosuroides* (black-grass) resistant tomesosulfuron+iodosulfuron. *Commun Agriculture Applied Biology Science* **74**(2), 479–488.

Oad, F.C., Siddiqui, M.H. & Buriro, U.A. 2007. Growth and yield losses due to different weed densities. *Asian Journal of Plant Sciences* **6**(1), 173–176. doi: https://doi.org/10.3923/ajps.2007.173.176

Oerke, E.C. 2006. Crop losses to pests. *Journal of Agricultural Science* **144**(1), 31–43.

Orson, J.H. & Harris, D. 1997. Technical and Financial Impact of Herbicide Resistant black-grass (*Alopecurus myosuroides*) on individual farm businesses in England. Proceedings of the 1997 Brighton Crop Protection Conference. *Weeds* **3**, 1127–1132.

Orson, J.H. & Thomas, M.R. 2001. Impact of generic herbicides on current and future weed problems and crop management. *Proceedings of Brighton Crop Protection Conference Weeds*, pp. 123–132.

Pilipavičius, V. 2012. *Herbicides in Winter Wheat of Early Growth Stages Enhance Crop Productivity, Herbicides – Properties, Synthesis and Control of Weeds*. Dr. Mohammed Nagib Hasaneen (Ed.), ISBN: 978-953-307-803-8, In Tech, Available from: http://www.intechopen.com/books/herbicides-properties-synthesis-

Ramsey, F.L. & Schafer, D.W. 1997. The Statistical Sleuth: A Course in Methods of Data Analysis. Belmont, CA: Duxbury, pp. 91–97.

Shehzad, M.A., Maqsood, M., Anwar-ul-Haq, M. & Niaz, A. 2012. Efficacy of various herbicides against weeds in wheat (*Triticum aestivum* L.). *African Journal of Biotechnology* **11**(4), 791–799. doi: https://doi.org/10.5897/AJB11.3274

Singh, R.D. & Gosh, A.K. 1992. Evaluation of herbicides for control of wildoat(*Avena ludoviciana*) in wheat (Triticum species). *Indian Journal of Agronomy* **37**, 327–331.

Tanji, A. & Boutfirass, M. 2018. Effective preemergence herbicides for rigid ryegrass(*Lolium rigidum* Gaud.) control in irrigated bread wheat(*Triticum aestivum* L.). *Journal of Agricultural Science* **10**(4), 79–85. doi: https://doi.org/10.5539/jas.v10n4p79

Thomas, C.G. & Yaduraju, N.T. 2000. Comparative growth and competitivenessof winter wild oats (*Avena sterilis* ssp. *ludoviciana*) and wheat (*Triticum aestivum*). *Indian Journal of Weed Science* **32**, 129–134.

Tomlin, C.D.S. 1994. *The Pesticide Manual*. 10th ed. Bath, UK: The Bath Press, 196 pp.

Torra, J., Cirujeda, A., Taberner, A. & Recasensm, J. 2010. Evaluation of herbicides to manage herbicide-resistant corn poppy (*Papaver rhoeas*) in winter cereals. *Crop Protection* **29**(7), 731–736.

Vouzounis, N.A. & Americanos, P.G. 1995. Residual activity of linuron and pendimethalin determined by bioassays in field trials. Agricultural Research Institute, Ministry of Agriculture, Natural Resources and the Environment, Nicosia, Cyprus. *Technical Bulletin* **169**, 3–7.

Agronomy Research **17**(6), 2399–2416, 2019
https://doi.org/10.15159/AR.19.218

Entrepreneurship education, entrepreneurship competencies and entrepreneurial activities of alumni: A comparison between the engineering and other graduates of Estonian University of Life Sciences

A. Põder[1,2,*], K. Lemsalu[1,3], M. Nurmet[1,3] and J. Lehtsaar[1]

[1]Estonian University of Life Sciences, Institute of Economics and Social Sciences, Kreutzwaldi street1a, EE51006 Tartu, Estonia
[2]Pennsylvania State University, The Northeast Regional Center for Rural Development, 207C Armsby building, University Park, PA 16802, USA
[3]Tartu University, Faculty of Social Sciences, School of Economics and Business Administration, J. Liivi street 4, EE50409 Tartu, Estonia
[*]Correspondence: anne.poder@emu.ee

Abstract. Entrepreneurial mind-set, knowledge and skills to recognise opportunities and implement ideas are vital competences for achieving success in the midst of rapid global changes. The main purpose of the entrepreneurship education is to foster those competencies. The present paper focuses on the role of the university education in developing various entrepreneurship competences, and the share of entrepreneurs among the alumni. The aim is more specifically to examine the relationship between entrepreneurship education and entrepreneurship competence development in university and the later entrepreneurial activities of the engineering alumni. The analysis is based on a questionnaire survey of alumni entrepreneurship conducted in 2016 as a part of a programme 'Edu ja Tegu- Development of entrepreneurial education throughout all educational levels'. *Chi-square tests, t-tests* are used to compare the engineering alumni of Estonian University of Life Sciences with graduates from other fields. The overall share of entrepreneurs among the engineering alumni was 35.6%. The entrepreneurial activities were impacted by the time of graduation. It had also impact of whether the graduates had received entrepreneurship courses during their studies. In comparison with other alumni, the engineering graduates assessed that their university education helped them develop significantly better problem-solving skills, critical thinking, self- evaluation skills, ability to develop new ideas and solutions and leadership skills and obtained significantly less entrepreneurial and financial knowledge during their studies. However, in case of engineering alumni, entrepreneurship education did not have significant impact on their entrepreneurial activities and assessments of competences, thus indicating that other factors are in play.

Key words: engineering education, entrepreneurship competencies, entrepreneurship education, graduates, university.

INTRODUCTION

Many studies have argued that providing entrepreneurial learning opportunities in universities and secondary schools has a favourable effect on entrepreneurship and innovation (Peterman & Kennedy, 2003; Kuratko, 2005; Van Praag & Versloot, 2007). The promotion of entrepreneurship is seen as a crucial source of economic growth and the educational system is more and more committed to honing entrepreneurial skills of the students. This can be illustrated by the prioritisation of the entrepreneurship education and training and its role in supporting business growth by the European Commission (2013) in 'The Entrepreneurship 2020 Action Plan'. Universities are increasingly seen as central actors, whose role is to contribute to entrepreneurship and economic development and provide their students with the skills they need to create and lead technology-rich entrepreneurial ventures (Barr et al., 2009).

Entrepreneurship has been identified by the European Commission as one of the eight key competences necessary for every citizen in modern society (Council Recommendations … 2018). Entrepreneurship as a competence can be understood as a transversal set of knowledge, skills and attitudes that enable to act upon opportunities and ideas and to turn those into action while creating value for others (Bacigalupo et al., 2016). Gibb (2008) emphasizes that those behaviours, skills and attributes help individuals and organisations to create and to adjust to changes in uncertain and complex situations.). The competence is based on '*creativity, critical thinking, problem solving, taking initiative and perseverance and the ability to work collaboratively'* (European Commission 2019, p. 13).

Entrepreneurship education describes the methodological approaches, educational content and activities that address the development of students' competences for entrepreneurial value creation (Moberg et al., 2014). Fayolle et al. (2006) emphasise that entrepreneurship education is pedagogy or a process that does not exclusively focus on new business creation, but on the development of specific attitudes and skills, including personal qualities. Education provides the opportunity to practice those behaviours (Gibb, 2008). Kirby (2004) argues that the entrepreneurship education should not focus only on new venture creation or small business management, but on the development of particular set of skills, attributes and behaviours necessary for successful entrepreneurship. This sets it aside from business education that focuses more narrowly on business creation. The competences developed through entrepreneurship education should increase individuals' employability and venturing as well as complement application of professional competencies while working as an employee or being entrepreneur (Mets et al., 2017). Entrepreneurship education is expected to increase entrepreneurial intentions and entrepreneurial self-efficacy that refers to the ability to carry out various tasks and roles connected with entrepreneurship (Bae et al., 2014).

Many authors (Katz, 2003; Kuratko, 2005; Matlay & Carey, 2007; Fayolle, 2013; Nabi et al., 2017; Neck et al., 2018) have noted that in the recent decades the provision of entrepreneurship education has rapidly increased. This includes the significant growth of entrepreneurship education programmes in universities (Morris et al., 2013). This has been followed by increase in the research on the effects of the entrepreneurship education. An increasing body of research has focused on the linkages between the entrepreneurship education and entrepreneurial intentions of students (e.g. Pittaway & Cope, 2007; Bae et al., 2014; Karimi et al., 2016; Maresch et al., 2016). The meta-analyses by Martin et al. (2013) and Bae et al. (2014) conclude that there is a positive effect of entrepreneurship education to entrepreneurial intentions. However, as Pittaway & Cope (2007) emphasize, the link between entrepreneurship education and outcomes in terms of actual enterprise creation has been under-researched.

Duval-Couetil et al. (2012), Maresch et al. (2016) have noted that the research on the impact of entrepreneurship education on engineering students has been relatively limited, although the number of publications on the topic have grown rapidly in the last years, as demonstrated by the analysis of Reis et al. (2019). Traditionally engineering education has focused on theoretical knowledge and prescribed content delivery, and less on entrepreneurial mindsets and creativity (Täks et al., 2016). However, it is acknowledged that there is a need to change the educational practices with putting more emphasis on developing students' creativity, innovativeness, mindsets and attitudes (Täks et al., 2014).

A review by Reis et al. (2019) on the research trends in engineering entrepreneurship education summarises that the effect of entrepreneurship education on entrepreneurial intentions and definition of entrepreneurship education have been the topics that dominate research. For example, Maresch et al. (2016) compared the impact of entrepreneurship education on entrepreneurial intentions of engineering and sciences students with outcomes in the group of business students. Their results demonstrated that entrepreneurship education had a positive effect on the entrepreneurship intentions in both groups, but also indicated to a potential 'Matthew effect', where business students with their prior background and education in business may benefit more from the entrepreneurship education. The positive impact of entrepreneurship education on intentions of engineering students was also suggested in the studies by Souitaris et al. (2007), Barba-Sánchez & Atienza-Sahuquillo (2017). Duval-Couetil et al. (2012) measured the outcomes of entrepreneurship education in terms of students' attitudes and perceptions, including self-evaluation of skills and abilities connected with entrepreneurship. The analysis of the latter demonstrated that engineering students who had received entrepreneurship education evaluated fifteen skills related to venturing and technology self-efficacy, and their general traits such as risk tolerance and ability to evaluate business ideas, significantly higher than those who had not received entrepreneurship education.

The outcomes of entrepreneurship education in terms of proceeding from intention to action by a subsequent enterprise creation has received more limited attention. One example is a study by Menzies & Paradi (2003), who compared groups of Canadian engineering graduates and demonstrated significantly higher business ownership rate after the graduation among those, who had received elective entrepreneurship courses. The results also indicated that those graduates established their enterprises sooner after the graduation in comparison with those who had not received entrepreneurship courses. An example of a longitudinal study on graduate entrepreneurship is provided by Matlay (2008), whose results indicated a positive relationship between entrepreneurship education and outcomes as graduates interested in entrepreneurship at the time of university studies progressed from self-employment to SME ownership and partnership in the following ten years.

The present research aims to contribute to the filling of the research gaps in research on the actual entrepreneurial activities of the graduates, and on the role entrepreneurship education played in the development of entrepreneurship competences. The analysis is based on a cross-sectional study of Estonian University of Life Sciences' alumni. The university is in the process of reforming its entrepreneurship education offering, and the main motivation for the study was to collect information on the entrepreneurship activities of graduates, because the university has not studied this before, and to collect feedback on the competences developed in order analyse the outcomes and shortcomings of education provided, and potential for improvement.

The main focus of the present analysis in on the engineering alumni and their entrepreneurship outcomes as they are expected to be the main creators of high growth entrepreneurship and technological innovation. Also, as entrepreneurship education has historically not been highly prioritised in the engineering curricula of particular university, the entrepreneurship rate of the alumni is a question of interest for the university. The main objective of the present research is to examine the relationship between entrepreneurship education and development of entrepreneurship competences in the university and the later entrepreneurial activities of the engineering alumni. The research questions are as follows:

- What is the share of entrepreneurs among engineering alumni in comparison with alumni of other fields?
- Did engineering graduates receive entrepreneurship education during their university studies and how did this affect their entrepreneurial activities later?
- How did the entrepreneurship education impact development of the entrepreneurship competencies of engineering graduates?
- How did engineering graduates who become entrepreneurs evaluate the development of entrepreneurship competences during their university studies in comparison with non-entrepreneurs?

The present paper is divided into four section. The introduction is followed by overview on materials and methods in the second section. The third section discusses the main results. The conclusions are presented in the fourth section.

MATERIALS AND METHODS

The present research is based on a cross-sectional study conducted in 2016. The data used was collected with an alumni survey that was part of the program 'Edu Tegu– Development of entrepreneurial education throughout all educational levels'. The entrepreneurial education programme was initiated by Estonian Ministry of Education and Research and was co-funded by the European Social Fund of the European Union. The present analysis focuses on the data collected from the graduates from Estonian University of Life Sciences (EULS). EULS is fourth in size among six Estonian public universities. With teaching and research going on in variety of fields representing both STEM fields and social sciences (economics), data from the alumni provides a good opportunity for comparing engineering graduates to the other fields.

The survey was conducted as a web-based questionnaire survey. The overall aim was to collect information about the graduates' entrepreneurial activities, the entrepreneurship education they received during their studies, and on their self-evaluation of different entrepreneurship competencies obtained during their studies, assessments on what kind of knowledge and skills should be emphasised more in curricula. For the university, this was an important feedback on the strengths and shortcomings of the education provided and on the activities of the students after their graduation. The sample consisted of the EULS graduates from the years 1951 to 2016. In total, the link to the survey was disseminated to the e-mail address of 6,496 persons. The study made use of the contacts of the graduates that registered for a university reunion event, university's information system's data on personal email addresses and on advertising the study in university's website and social media. 1,457 responses were obtained (Põder et al., 2016), however, some of those responses are also partial with missing data in case of some of the variables.

In the survey respondents were asked to name their field of studies. In the analysis engineering graduates refers to respondents who graduated from agricultural and production engineering, husbandry engineering and ergonomics, energy engineering and technology curricula and attended the university's present-day Institute of Technology or its predecessor Faculty of Agricultural Mechanisation. The other alumni refer to the graduates of agriculture, forestry, fisheries; life sciences; veterinary science and animal husbandry; business and administration and other curricula of other institutes of the EULS. The field of studies question was completed in case of 1,417 respondents that are used in this analysis. The number of engineering graduates in the analysis was 195 (13.4% of the respondents). The engineering graduates' average age at the time of the study was 41.31 years [$SD = 14.74$; for other alumni $M = 39.45$; $SD = 12.88$; $t(1{,}133) = 1.651$, $p = 0.099$]. Share of men among engineering graduates was 93% [35.6% among graduates of other institutes; $\chi^2(1, N = 1{,}131) = 180.96$, $p < 0.001$]. The higher share of men among the engineering students is common across countries (e.g. as reported in the studies of Menzies & Paradi 2003; Duval-Couetil et al., 2012). One question of interest in the study was the change in the provision of entrepreneurship education over time. Thus, for the analysis, respondents are also divided into two groups on the basis of their graduation time: from 2006 to 2016 (ten years from the time of study at 2016 and a period of relative stability in terms of curriculum reforms) and before 2006. 60.6% of engineering graduates and 61.6% of other graduates had graduated from the university between 2006–2016 [$\chi^2(1, N = 1{,}404) = 0.016$, $p = 0.898$].

In the survey, entrepreneurs were operationalized as graduates who were self-employed or owners and managers of commercial enterprises or non-profit organisations. Entrepreneurship education was surveyed with the question on whether the respondents participated in any entrepreneurship courses during their university studies. Entrepreneurship competencies was studied with a list of 22 competencies (Table 2). The list was compiled on the basis of literature and analysis of learning outcomes of EULS entrepreneurship courses and supplemented by the feedback from entrepreneurship lecturers. Respondents were asked to evaluate in a Likert-type of scale of 5 (5- certainly yes …. 1- certainly not) whether their studies helped them to obtain those competencies.

Chi-square tests was used to compare the share of entrepreneurs among engineering and other alumni and if different groups of alumni had received entrepreneurship education. *Independent sample t-tests* were used for studying mean age upon the person became an entrepreneur and compare the mean assessment on entrepreneurship competences between different groups (Table 2 to Table 6). The data was checked for the assumptions of *chi-square* and *t-tests* (using 95% confidence interval). There were no violations in the assumptions of *chi-square* tests. In the *independent sample t-tests* presented in Table 2, the homogeneity of variance assumption was broken in case of 7 comparisons, so for those comparison *Welch t-test* is reported in the table. Also, in the Tables 3 to 6, if the variance between the two comparison groups was unequal, results of *Welch t-test* is presented.

RESULTS AND DISCUSSION

Share of entrepreneurs

The share of entrepreneurs among all respondents at the time of the study in 2016 was 31%. That is relatively close to the current enterprise ownership rate reported, for example, in the Menzies & Paradi's (2003) study. The overall share of entrepreneurs among the engineering alumni was 35.6%; in other alumni 30.2%, but the difference not statistically significant [$\chi^2(1, N = 1{,}389) = 2.23$, $p = 0.135$]. However, the differences were significant, if the time of graduation and average age of the graduate at the time when the enterprise was established were considered. At the time of study, the share of entrepreneurs among the engineering alumni that graduated before 2006, was significantly higher [53.9% vs. 39.3%, Fig. 1, $\chi^2(1, N = 189) = 20.25$, $p < 0.001$]. Although the share of entrepreneurs was lower among the other alumni, the association with the period of graduation before or after 2006 was also significant [$\chi^2(1, N = 1{,}187) = 32.08$, $p < 0.001$].

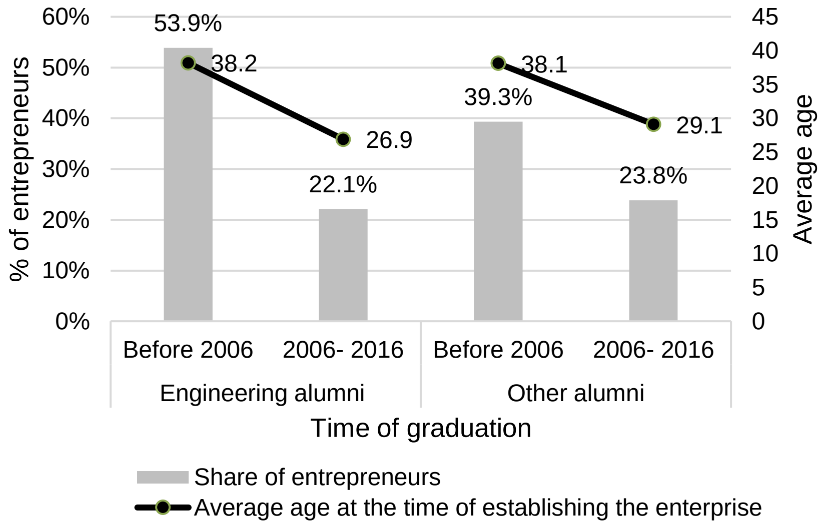


Figure 1. Share of entrepreneurs among the alumni and average age of the graduate at the time of establishment of the enterprise.

One possible explanation for the considerably higher share of entrepreneurs among engineering graduates is also the socioeconomic context of the period. The group of those earlier graduates includes those, who worked as engineers in the 1990s that is the transition period from socialist economy to the market economy in Estonia. This time period is characterized by the collapse of previous economic relations and industrial restructuring and considerable changes in labour demand (Viira et al., 2009; Põder et al., 2017). The activities of EULS and the engineering education it provides has historically been strongly connected with agriculture, thus the developments in agriculture have had considerable impact on its graduates. With the decline and restructuring of Soviet era large scale industries and agro-industrial complexes the demand for engineers dropped and this drove necessity-based entrepreneurship by forcing the graduates previously employed in those industries and large-scale organisations set out on their own and become entrepreneurs. At the same time re-establishment of private entrepreneurship after Soviet period in which private entrepreneurship was officially forbidden in Estonia, the transition period offered also new entrepreneurship opportunities that also drove opportunity-based entrepreneurship.

The increase of entrepreneurs among the graduates over time indicates also to the time lag between the graduation and entrepreneurship activities discussed by Lüthje & Franke (2003) and Souitaris et al. (2007). However, the analysis of survey data also shows that on average the graduates became entrepreneurs in the age of 33.5 years [engineering graduates $M = 33.67$, $SD = 9.47$; other alumni $M = 33.47$, $SD = 9.32$; $t(356) = 0.149$, $p = 0.882$], but there is clear tendency that more recent graduates start their enterprises at younger and younger age. In both alumni groups respondents who graduated in the period of 2006–2016, were significantly younger when getting involved in entrepreneurship (Fig. 1). For engineering graduates the average age when becoming an entrepreneur was higher for those who had graduated before 2006 ($M = 38.17$, $SD = 9.17$) in comparison with graduates from period 2006 and later ($M = 26.92$, $SD = 4.78$), $t(55) = -6.19$, $p < 0.001$. The trend in similar in the group from other fields of study as earlier graduates started their entrepreneurial activities at a later age ($M = 38.06$, $SD = 9.47$) than the entrepreneurs in the group that graduated university after 2006 ($M = 29.12$, $SD = 6.79$), $t(260) = -9.33$, $p < 0.001$.

This could be explained by the increased integration of entrepreneurship education to secondary and higher education in Estonia that has been going on in the last decade (Täks et al., 2014; Raudsaar & Kaseorg, 2016). The educational efforts and increased public attention would provide the graduates with necessary skills and knowledge and encouragement for becoming an entrepreneur. But an additional factor that may explain the younger start-up age is another institutional change for entrepreneurship start-up process. Since 2011, the legal requirements for the share capital while setting up a private limited company have been relaxed in Estonia and this has played the role encouraging enterprise creation (Põder et al., 2017).

Entrepreneurship education

Share of alumni, who had received entrepreneurship courses during their university studies, was significantly lower among engineering alumni (44.5%) in comparison with in other alumni (57.9%), [$\chi^2(1, N = 1{,}147) = 9.232$, $p = 0.002$].This differs from the results of Maresch et al. (2016), in case of which engineering graduates had received more entrepreneurship courses than business students.

As more attention is paid to entrepreneurship education, in case of engineering alumni who graduated after 2006, 59.8% had entrepreneurship courses in their curricula in comparison with 24.2% of engineering alumni, who had graduated before 2006 [$\chi^2(1, N = 158) = 17.216$, $p < 0.001$]. The trend of increase of entrepreneurship courses for engineering students is similar to that reported by Menzies & Paradi (2003).

The significant difference characterised also other alumni as 42.9% of those graduating before 2006 reported entrepreneurship courses, while in case of those who graduated later in 2006–2016, their share had increased to 67.6% [$\chi^2(1, N = 979) = 57.919, p < 0.001$]. This demonstrates the expansion of entrepreneurship education in higher education as in the last decade several programs and governmental efforts have concentrated on increasing the number of entrepreneurship courses in different education levels in Estonia as well as creating start-up programs and competitions and other similar opportunities (Täks et al., 2014).

In case of engineering alumni, entrepreneurship courses did not impact entrepreneurial activities (43.8% of engineering alumni entrepreneurs had taken entrepreneurship courses vs. 45.8% of those who were not entrepreneurs, Table 1), $\chi^2(1, N = 160) = 0.679, p = 0.795$. The comparison by the time of the graduation shows that 26.3% of entrepreneurs among the engineering graduates from the period before 2006 had received entrepreneurship courses. In case of non-entrepreneurs, 20.8% of them had received entrepreneurship courses, but the difference with entrepreneurs was not significant [$\chi^2(1, N = 62) = 0.241, p = 0.623$]. For the engineering alumni, who graduated in 2006 to 2016, 70.8% of entrepreneurs and 55.6% of non-entrepreneurs had received entrepreneurship education, however the difference was also not significant [$\chi^2(1, N = 87) = 1.687, p = 0.194$].

Table 1. Share of alumni, who received entrepreneurship education during their university studies

Time of graduation		Engineering alumni	Other alumni	TOTAL
Before 2006	Entrepreneurs	26.3%	52.3%	46.9%
	Non- entrepreneurs	20.8%	35.4%	35.1%
	Total	24.2%	42.9%	40.5%
	p	n.s	**	**
2006 to 2016	Entrepreneurs	70.8%	78.1%	77.2%
	Non- entrepreneurs	55.6%	63.7%	62.8%
	Total	59.8%	67.5%	66.7%
	p	n.s	**	***
TOTAL	Entrepreneurs	43.8%	65.4%	61.8%
	Non- entrepreneurs	45.8%	54.2%	53.9%
	Total	44.5%	57.9%	56.7%
	p	n.s	**	**

**p < 0.05; **p < 0.01; ***p < 0.001; n.s- not significant.*

However, in case of other graduates, entrepreneurship courses had significant impact (65.4% of graduates who became entrepreneurs had received entrepreneurship courses as part of their studies vs. 54.2% of those who were not entrepreneurs, $\chi^2(1, N = 980) = 10.920, p < 0.01$). The comparison by the time of graduation shows the among those, who graduated before 2006, 52.3% of entrepreneurs had received entrepreneurship education in comparison with 35.4% of non-entrepreneurs who had entrepreneurship courses [$\chi^2(1, N = 343) = 9.859, p < 0.01$). Similar significant difference occurred also in the group of other alumni, who graduated in the period of 2006 to 2016.

Thus, while in non-engineering alumni entrepreneurship education had positive impact on later entrepreneurial activities, this was not the case for engineering alumni. In the period before 2006 more than half of engineering alumni had become entrepreneurs, despite of the fact that most of them had not received any entrepreneurship education. For those, who had received entrepreneurship education, it failed to have significant impact on whether they become entrepreneurs or not. It can be assumed that in case of the engineering alumni the choice to become an entrepreneur was impacted by other factors than entrepreneurship education. In the present study the group of non-engineering alumni did not include only business students, but also other fields of studies, so our results are not comparable with those of Maresch et al. (2016) in one-on-one. However, with the lack of effect of entrepreneurship education in engineering students, but impact on other graduates, it is possible that there is a similar effect as suggested in their research, where different groups (in their case business students) may benefit more from the entrepreneurship education.

Assessments on the development of entrepreneurship competencies

In the questionnaire survey the graduates were asked to assess 22 different knowledge and skills connected with entrepreneurship in a Likert type of scale of 5 (Table 2). Overall, the highest scores were given to the development of ability to continuously work of self-improvement, independence, oral and written expression skills and communications skills. Entrepreneurial knowledge and financial knowledge received the lowest scores. The engineering alumni on average gave higher scores to the competences studied.

Table 2. Comparison of the assessment on how did university studies help to develop the following competences

	Engineering alumni		Other alumni		*t-value*	*df*	*p*
	M	*SD*	*M*	*SD*			
Continuous self-improvement	4.09	0.74	4.08	0.74	0.059	1,134	n.s
Independence	4.10	0.89	3.98	0.88	1.64	1,129	n.s
Oral and written expression skills	3.88	0.87	3.94	0.87	-0.843	1,134	n.s
Communication skills	3.85	0.91	3.89	0.89	-0.505	1,130	n.s
Teamworking	3.91	0.77	3.85	0.89	0.952	230.74	n.s
Planning skills	3.85	0.81	3.83	0.85	0.178	1,126	n.s
Ability to work on long-term goals	3.79	0.95	3.84	0.88	-0.681	1,125	n.s
Problem-solving skills	4.09	0.77	3.74	0.95	5.121	240.65	***
Critical thinking	3.94	0.83	3.71	0.89	3.09	218.04	**
Critical evaluation of own skills	3.89	0.81	3.72	0.88	2.447	222.82	*
Ethical behaviour	3.84	0.86	3.73	0.92	1.410	210.86	n.s
Self-confidence	3.75	0.84	3.72	0.87	0.385	1,126	n.s
Need for achievement	3.61	0.93	3.56	0.97	0.542	1,123	n.s
Networking ability	3.59	0.96	3.49	1.04	1.198	1,124	n.s
Developing new ideas and solutions	3.80	0.83	3.42	0.95	5.162	228.54	***
Creativity	3.68	0.98	3.43	0.96	3.058	1,121	**
Initiative	3.53	0.91	3.42	0.97	1.292	1,122	n.s
Risk taking	3.48	1.00	3.34	0.99	1.667	1,125	n.s
ICT skills	3.39	1.09	3.35	1.09	0.359	1,125	n.s
Leadership skills	3.37	0.99	3.17	1.05	2.214	1,130	*
Financial knowledge	2.70	1.01	3.13	1.18	-4.800	230.31	***
Entrepreneurial knowledge	2.87	1.06	3.10	1.16	-2.265	1,122	*

**$p < 0.05$; **$p < 0.01$; *** $p < 0.001$; n.s.- not significant.*

The comparison between engineering alumni and other alumni indicated that in case of 8 competences the difference between the scores of two groups were statistically significant. In comparison with other alumni engineering alumni gave considerably higher assessments to the development of problem-solving skills, critical thinking, critical evaluation of own skills, ability to develop new ideas and solutions, but also to creativity and leadership. The scores were significantly lower in case of entrepreneurial knowledge and financial knowledge. On the basis of the results in can be concluded that in the opinion of the engineering alumni, the university education generally provided them with a mix of skills critical for engineering profession and for acting upon different opportunities. Given the nature of engineering work, it can be expected that they developed better competences than other alumni in problem solving, critical thinking, and finding new solutions as this also requires creativity, but interesting aspect was also the better outcomes in leadership skills.

T-tests were also used to study whether entrepreneurship education had impact on the evaluations on the development of various competencies (Table 3 and 4). In case of the engineering alumni, entrepreneurship courses were connected only with three competencies: entrepreneurial knowledge, financial knowledge and ICT skills. Engineering alumni, who had attended entrepreneurship courses, gave statistically significantly higher assessments on whether the studies helped to acquire those skills and knowledge.

Table 3. Mean scores of engineering alumni on the assessments on how did university studies help to develop the following competences on the basis of whether they received entrepreneurship courses (EC) or not

	EC		No EC		*t-value*	*df*	*p*
	M	*SD*	*M*	*SD*			
Continuous self-improvement	4.12	0.75	4.03	0.73	-0.492	156	n.s
Independence	3.99	0.94	3.96	0.85	-0.400	155	n.s
Oral and written expression skills	4.00	0.88	3.85	0.85	1.019	156	n.s
Communication skills	3.93	0.89	3.82	0.92	1.179	155	n.s
Teamworking	3.91	0.82	3.76	0.72	0.060	156	n.s
Planning skills	3.91	0.79	3.73	0.82	0.168	155	n.s
Ability to work on long-term goals	3.92	1.04	3.72	0.88	-0.716	133.16	n.s
Problem-solving skills	3.78	0.82	3.68	0.72	-0.566	156	n.s
Critical thinking	3.74	0.80	3.66	0.86	0.100	155	n.s
Critical evaluation of own skills	3.73	0.73	3.69	0.86	1.113	156	n.s
Ethical behaviour	3.71	0.86	3.75	0.85	1.155	151	n.s
Self-confidence	3.81	0.90	3.59	0.79	-0.265	153	n.s
Need for achievement	3.64	0.92	3.44	0.94	-0.680	155	n.s
Networking ability	3.59	0.99	3.32	0.95	0.321	155	n.s
Developing new ideas and solutions	3.46	0.83	3.36	0.84	-0.309	156	n.s
Creativity	3.41	0.96	3.43	0.99	-0.421	154	n.s
Initiative	3.49	0.86	3.32	0.95	-0.232	154	n.s
Risk taking	3.35	0.96	3.32	1.04	-0.820	154	n.s
ICT skills	3.61	0.96	3.00	1.16	2.595	155	*
Leadership skills	3.25	0.99	3.04	1.00	0.284	155	n.s
Financial knowledge	3.53	0.97	2.57	1.00	2.952	155	**
Entrepreneurial knowledge	3.49	0.86	2.54	1.09	4.306	154	***

*$**p < 0.05$; $**p < 0.01$; *** $p < 0.001$; n.s.- not significant.*

In the assessments of the alumni from other fields (Table 4), the entrepreneurship education had considerably more impact. Respondents from other fields gave significantly higher scores to more than half of the competences in the list (to 12 competences out of 22). Besides the entrepreneurial and financial knowledge and ICT skills that were significantly different also in case of engineering alumni, such competences as networking skills, self-confidence, leadership skills, ability to work for long-term goals, need for achievement, planning, taking initiative, written and oral expression skills, teamwork were impacted by entrepreneurship courses the alumni of other fields had received during their studies. Thus, it can be assumed that while those skills are typically emphasised as transversal competences that all courses in the university should help to develop, the entrepreneurship education has a significant role in contributing to the development of certain skills.

Table 4. Mean scores of other alumni on the assessments on how did university studies help to develop the following competences on the basis of whether they received entrepreneurship courses (EC) or not

	EC		No EC		*t-value*	*df*	*p*
	M	*SD*	*M*	*SD*			
Continuous self-improvement	4.12	0.73	4.03	0.75	1.771	964	n.s
Independence	3.99	0.87	3.96	0.89	0.558	962	n.s
Oral and written expression skills	4.00	0.86	3.85	0.87	2.705	965	**
Communication skills	3.93	0.89	3.82	0.89	1.922	961	n.s
Teamworking	3.91	0.84	3.76	0.93	2.507	813.79	*
Planning skills	3.91	0.83	3.73	0.84	3.291	958	**
Ability to work on long-term goals	3.92	0.86	3.72	0.89	3.428	841.06	**
Problem-solving skills	3.78	0.93	3.68	0.97	1.683	996	n.s
Critical thinking	3.74	0.88	3.66	0.90	1.443	957	n.s
Critical evaluation of own skills	3.73	0.89	3.69	0.87	0.840	958	n.s
Ethical behaviour	3.71	0.95	3.75	0.87	-0.654	889.66	n.s
Self-confidence	3.81	0.85	3.59	0.88	3.941	960	***
Need for achievement	3.64	0.96	3.44	0.97	3.092	955	**
Networking ability	3.59	1.04	3.32	1.01	4.009	956	***
Developing new ideas and solutions	3.46	0.98	336	0.91	1.473	899.02	n.s
Creativity	3.41	0.97	3.43	0.93	-0.209	954	n.s
Initiative	3.49	0.97	3.32	0.94	2.740	955	**
Risk taking	3.35	1.01	3.32	0.97	0.516	958	n.s
ICT skills	3.61	0.98	3.00	1.15	8.821	957	***
Leadership skills	3.25	1.05	3.04	1.04	3.140	962	**
Financial knowledge	3.53	1.08	2.57	1.07	13.642	961	***
Entrepreneurial knowledge	3.49	1.06	2.54	1.06	13.581	955	***

**p < 0.05; **p < 0.01; *** p < 0.001; n.s.- not significant.*

Another question of interest in the study was whether those, who later became entrepreneurs, have different opinion on how did university education contribute to the development of various competences. Table 5 provides comparisons for the engineering graduates. As it can be seen from the comparisons, the entrepreneurs among the engineering graduates did not report significantly different development of various skills and knowledge. Entrepreneurs gave statistically different assessments to only two competences. Networking ability received higher scores and ICT skills lower scores from entrepreneurs than non-entrepreneurs among engineering alumni (Table 5).

Networking is connected with skills for social interaction and with creation, maintenance and using of social relationships to advance individual goals (Morris et al., 2013). While the other competences did not make difference for the engineering alumni, it seems that those, who built up relationships during their university studies, were in a better position to use those for their entrepreneurship activities. As one of our takes from the analysis has been that the entrepreneurship activities of engineering graduates were also induced by the economic climate and contraction and restructuring of large-scale industries, it can be expected that those individuals with good networks were in particularly favourable position for accessing various resources during a period of economic and social turmoil.

Table 5. Mean scores of engineering alumni on the assessments on how did university studies help to develop the following competences on the basis of whether they are entrepreneurs

	Entrepreneurs		Non-entrepreneurs		*t-value*	*df*	*p*
	M	*SD*	*M*	*SD*			
Continuous self-improvement	4.08	0.67	4.11	0.80	-0.256	141.05	n.s
Independence	4.13	1.02	4.13	0.80	0.013	141	n.s
Oral and written expression skills	3.86	0.96	3.93	0.80	-0.467	142	n.s
Communication skills	3.84	0.97	3.85	0.84	-.0057	141	n.s
Teamworking	3.90	0.85	3.91	0.69	-0.068	142	n.s
Planning skills	3.83	0.75	3.89	0.79	-0.475	141	n.s
Ability to work on long-term goals	3.81	1.03	3.81	0.89	-0.023	139	n.s
Problem-solving skills	4.11	0.82	4.10	0.76	0.093	142	n.s
Critical thinking	3.94	0.87	3.99	0.83	-0.355	141	n.s
Critical evaluation of own skills	3.86	0.87	3.90	0.78	-0.318	142	n.s
Ethical behaviour	3.87	0.87	3.81	0.85	0.430	138	n.s
Self-confidence	3.76	1.10	3.76	1.02	0.039	139	n.s
Need for achievement	3.56	0.91	3.66	0.89	-0.702	141	n.s
Networking ability	3.79	1.03	3.48	0.84	2.031	141	*
Developing new ideas and solutions	3.84	0.78	3.80	0.87	0.276	142	n.s
Creativity	3.84	0.94	3.55	0.99	1.756	140	n.s
Initiative	3.68	0.94	3.43	0.85	1.662	140	n.s
Risk taking	3.56	1.01	3.42	0.98	0.820	140	n.s
ICT skills	3.19	1.10	3.65	1.02	-2.578	141	*
Leadership skills	3.56	1.04	3.28	0.88	1.704	121.54	n.s
Financial knowledge	2.79	1.09	2.63	0.90	1.008	141	n.s
Entrepreneurial knowledge	2.98	1.16	2.79	0.96	1.098	140	n.s

$^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$; n.s.- not significant.

The lack of difference in the engineering alumni scores indicates relatively uniform opinions on what kind of education the university provided them with. Engineering alumni developed same kind of entrepreneurship competencies throughout their studies regardless of whether they later become entrepreneurs or not. This could indicate that the competences developed during studies should be sufficient base if the graduate later decides to become an entrepreneur. Also, those who later become entrepreneurs did not necessarily seek out the development of very specific competences during the studies.

The entrepreneurs from other alumni also displayed same kind of patterns in their assessments (Table 6). But besides those two competences, also problem solving, risk taking, leadership and financial knowledge received higher scores from entrepreneurs than non-entrepreneurs.

Table 6. Mean scores of other alumni on the assessments on how did university studies help to develop the following competences on the basis of whether they are entrepreneurs

	Entrepreneurs		Non-entrepreneurs		*t-value*	*df*	*p*
	M	*SD*	*M*	*SD*			
Continuous self-improvement	4.11	0.74	4.08	0.73	0.656	910	n.s
Independence	3.95	0.96	4.01	0.82	-0.954	908	n.s
Oral and written expression skills	3.90	0.90	3.97	0.84	-1.078	912	n.s
Communication skills	3.89	0.89	3.91	0.88	-0.394	910	n.s
Teamworking	3.82	0.91	3.87	0.86	-0.783	909	n.s
Planning skills	3.80	0.89	3.86	0.82	-0.984	571.88	n.s
Ability to work on long-term goals	3.87	0.90	3.83	0.88	0.565	906	n.s
Problem-solving skills	3.83	0.96	3.70	0.93	2.020	913	*
Critical thinking	3.71	0.95	3.74	0.86	-0.438	904	n.s
Critical evaluation of own skills	3.75	0.92	3.73	0.87	0.269	905	n.s
Ethical behaviour	3.68	0.93	3.77	0.90	-1.457	901	n.s
Self-confidence	3.74	0.87	3.71	0.86	0.554	906	n.s
Need for achievement	3.59	0.96	3.57	0.97	0.334	903	n.s
Networking ability	3.60	1.04	3.44	1.03	2.308	903	*
Developing new ideas and solutions	3.48	0.93	3.40	0.95	1.276	909	n.s
Creativity	3.47	0.95	3.43	0.96	0.722	903	n.s
Initiative	3.51	0.97	3.38	0.96	1.836	905	n.s
Risk taking	3.44	1.02	3.30	0.97	1.991	907	*
ICT skills	3.31	1.12	3.39	1.07	-1.144	904	*
Leadership skills	3.31	1.03	3.08	1.05	3.129	908	**
Financial knowledge	3.26	1.21	3.07	1.65	2.322	907	*
Entrepreneurial knowledge	3.20	1.17	3.04	1.42	1.916	903	n.s

**p < 0.05; **p < 0.01; ***p < 0.001; n.s- not significant.*

Problem solving skills, networking and leadership skills were among those competences that other alumni had scored significantly lower in comparison with engineering alumni (Table 2), and those skills seem to be particularly critical for encouraging entrepreneurship among the graduates from other fields. For networking ability and leadership as well as for financial knowledge and ICT, the entrepreneurship education made difference (Table 4). While the problem solving and risk-taking ability were important in becoming an entrepreneur, the entrepreneurship education did not build up particular skills during the entrepreneurship courses.

Limitations of the survey

The present research has several limitations as the analysis is relatively descriptive in its nature and the data collected with the questionnaire was based on the respondents' self-reports and not on the actual measurement of performance. This included reports on whether the graduates had received any entrepreneurship courses as part of their university studies. The shortcoming of the survey is that the questionnaire did not specify the amount of entrepreneurship courses, including the number of courses or credit hours in detail, or their specific content. The collection of detailed data on how many and which kind of courses were part of the particular curricula was beyond the scope of this survey, because the aim was to collect data on the activities of graduates from all programs spanning over several decades. This included dozens of different study programs and curricula that have been subjected to profound changes. The changes over the span of time, including in the definition of credit hours, make it impossible to measure the workload, exact number of courses or detailed content of the course reliably on the basis of the graduates' recall.

Also, as entrepreneurs were operationalised on the basis of the question on whether the respondent was a sole proprietor and owner and manager of a commercial enterprise or non-profit; thus it is impossible to differentiate on whether they fit criteria for a classic Schumpeterian definition of entrepreneur or a regular everyday business owner.

CONCLUSIONS

Engineering alumni is expected to be in the forefront of a knowledge-based society by contributing to the new venture creation and technological developments and solutions for modern problems. With just over a third of engineering alumni involved in entrepreneurial activities, the research on the role of entrepreneurship education in their entrepreneurship activities provides valuable feedback on how to improve the attainment of knowledge and skills necessary for university students later in their life.

The entrepreneurial activities were impacted by the time of graduation that also had impact of whether the graduates had received entrepreneurship courses during their studies. We associate the significantly higher share of entrepreneurs among engineering graduates from the period before 2006 with the considerable economic restructuring that was taking place in the transition period. Part of this process was the collapse of Soviet era argo-industrial complexes and contraction of newly privatized industrial and agricultural enterprises (Viira et al., 2009; Põder et al., 2017). This was accompanied by considerable decrease in engineering jobs in the industry and agriculture. The ongoing economic and institutional changes created both opportunity and necessity-based entrepreneurship in the field of engineering that could explain the higher entrepreneurial activity of engineering graduates in comparison with other fields.

The socioeconomic context can also explain the lack of impact on entrepreneurship education on entrepreneurial activities of engineering alumni. While the share of engineering alumni who had received entrepreneurship education during their university studies was two times lower in comparison of alumni of other fields that graduated before 2006, the share of entrepreneurs was considerably higher in engineering alumni. Thus, they became entrepreneurs despite of lack of education in the field of entrepreneurship. This is also demonstrated by the assessments on the entrepreneurship competencies as the engineering alumni indicated that they had less financial knowledge and entrepreneurial knowledge than those in other alumni. While the entrepreneurship courses helped to build those competences, better skills in those areas did not impact whether the engineering graduates became entrepreneurs. This also indicates the possibility that the socioeconomic developments in their particular field were the primary drivers for entrepreneurship activities. For example, among those who graduated after 2006, the difference in the share of entrepreneurs between engineering and other alumni disappears.

The entrepreneurship education had more important role in developing different competencies in other alumni than in engineering graduates. However, the evaluation on the competences indicates that the engineering education of EULS provided a well-rounded development of transversal competences throughout the different subjects even without the entrepreneurship courses. With higher scores on most of competences studied in comparison with other alumni from different fields, the competences such as problem-solving abilities, critical thinking, creativity, development of new ideas and solutions served the engineering alumni well in solving the issues related with their entrepreneurship activity.

Another result that indicates to this direction mentioned in above, is the lack of significant differences between the scores of engineering alumni who had received entrepreneurship courses in comparison with those who had not participated in any. In the interpretation of this results, the limitations of the present study have to be considered. As the study does not provide information on the actual content and on how much entrepreneurship education was received, the lack of impact of entrepreneurship education on the development of competences of engineering alumni can be related with very limited access to entrepreneurship courses. As entrepreneurship courses have not traditionally been prioritized in the Estonian engineering education and less than half of engineering alumni had participated in any entrepreneurship courses, it is highly likely that the entrepreneurship education in the engineering programs consisted of a single obligatory course and/or a random elective course. In case of other alumni the entrepreneurship education increased the likelihood of them later becoming an entrepreneur and the other alumni included graduates from the fields (e.g. business and administration) which study programs have traditionally contained an integrated set of entrepreneurship courses. Thus, the actual content and volume of entrepreneurship education and how it impacts the entrepreneurial activities after the university graduation requires further research attention.

Typically, most of university graduates do not set up their enterprise right after finishing the university, but in somewhat older age after working as an employee and building up experience and network. Present results demonstrated that in the last decade the graduates have started to become entrepreneurs at younger and younger age. This could be explained by institutional changes in legislation and the effort of the government to encourage entrepreneurship by simplifying the administrative processes for setting up enterprises as well as by expansion of entrepreneurship education. But it also indicates that entrepreneurship education in higher education requires further attention from policymakers and researchers. When considerable share of graduates become entrepreneurs sooner after their university graduation as in previous decades, the entrepreneurship education they received will have more direct and quicker impact on their actual entrepreneurial activities.

Despite the methodological shortcomings of the present survey, we find that our results indicate to some useful implication for the further research. While the impact of entrepreneurship education on the formation of entrepreneurial intentions has been the subject of increased amount of research, the question of how does it translate into action and when, should receive more attention.

Another question that our study failed the address, is how the competences erode over time and which kind of competences turn out to be more critical for different paths later in life. This particular study looked back and asked the graduates to assess their education years after they received it. However, a longitudinal study would provide means to collect data on the students' views on their competences at the time of university studies and contrast those with assessments collected later in life and affected by experience of implementing those competences in real life. This kind of methodology would help to address the time lags between the university studies and entrepreneurial activity. Our study indicated decrease in the average age for entrepreneurship and we interpret that it is partially caused by increased access to entrepreneurship education, incubators, accelerators etc. that encourage entrepreneurship. But another question of interest is whether this results in better performance in comparison with entrepreneurs who build up experience and networks with working for a longer period as an employee in industry, before setting up their entrepreneurial endeavours.

Our interpretation of some of the results was tied to the institutional changes in the society. The future studies should account for the institutional context of the entrepreneurship activities and entrepreneurship education. Many studies have integrated the perception of social norms, societies' attitudes towards entrepreneurship into their study. However, in particular fields of economic activities, the rapid contraction or expansion of particular industry and its labour demand is likely to be more primary driver of entrepreneurship activities.

ACKNOWLEDGEMENTS. The alumni entrepreneurship survey carried out by Estonian University of Life Sciences was part of the program 'Edu Tegu- Development of entrepreneurial education throughout all educational levels' that is co-funded by the European Social Fund of the European Union.

REFERENCES

Bacigalupo, M., Kampylis, P., Punie, Y. & Van den Brande, G. 2016. EntreComp: The Entrepreneurship Competence Framework. Luxembourg: Publication Office of the European Union. https://ec.europa.eu/jrc/en/publication/eur-scientific-and-technical-research-reports/entrecomp-entrepreneurship-competence-framework Accessed 18.07.2019.

Bae, T.J., Qian, S. & Miao, C. 2014. The relationship between entrepreneurship education and entrepreneurial intentions: a meta-analytic review. *Entrep. Theory. Pract.* **38**(2), 217–254.

Barba-Sánchez, V. & Atienza-Sahuquillo, C. 2017. Entrepreneurial intention among engineering students: The role of entrepreneurship education. *Eur. Res. Manag. Bus. Econ.* **24**(1), 53–61.

Barr, S.H., Baker, T., Markham, S.K. & Kingon, A. 2009. Bridging the Valley of Death: Lessons Learned From 14 Years of Commercialization of Technology Education. *Acad. Manag. Learn. Edu.* **8**(3), 370–388.

Council Recommendation of 22 May 2018 on key competences for lifelong learning. Official Journal of the European Union. C 189/1. 4.6.2018, 1–13. https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32018H0604(01)&from=EN Accessed 01.05.2019

Duval-Couetil, N., Reed-Rhoads, T. & Haghighi, S. 2012. Engineering Students and Entrepreneurship Education: Involvement, Attitudes and Outcomes. *Int. J. Eng. Edu.* **28**(2), 425–435.

European Commission. 2013. Communication from the Commission to the European Parliament, the COUNCIL, the European Economic and Social Committee and the Committee of the Regions Entrepreneurship 2020 ACTION PLAN Reigniting the entrepreneurial spirit in Europe. COM/2012/0795. Brussels. http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:52012DC0795 Accessed 30.01.2017

European Commission. 2019. Key Competencies for Lifelong Learning. Directorate-General for Education, Youth, Sport and Culture. Luxembourg: Publication Office of the European Union.

Fayolle, A., Gailly, B. & Lassas-Clerc, N. 2006. Assessing the impact of entrepreneurship education programmes: A new methodology. *J. Eur. Ind. Train.* **30**(9), 701–720.

Fayolle, A. 2013. Personal views on the future of entrepreneurship education. *Entrep. Region. Dev.* **25**(7–8), 692–701.

Franke, N. & Lüthje, C. 2004. Entrepreneurial intentions of business students –A benchmarking study. *Int. J. Innov. Technol. Manag.* **1**(03), 269–288.

Gibb, A.A. 2008. Entrepreneurship and Enterprise Education in Schools and Colleges: Insights from UK Practice. *Int. J. Entrep. Edu.* **6**(2), 1–48.

Karimi, S., Biemans, H.J.A., Lans, T., Chizari, M. & Mulder, M.A.F. 2016. The Impact of Entrepreneurship Education: A Study of Iranian Students' Entrepreneurial Intentions and Opportunity Identification. *J. Small. Bus. Manag.* **54**(1), 187–209.

Katz, J.A. 2003. The chronology and intellectual trajectory of American entrepreneurship education: 1876–1999. *J. Bus. Venturing.* **18**(2), 283–300.

Kirby, D.A. 2004. Entrepreneurship education: can business schools meet the challenge? *Edu + Train.* **46**(8–9), 510–519.

Kuratko, D. 2005. The emergence of entrepreneurship education: Development, trends, and challenges. *Entrep. Theory. Prac.t* **29**(5), 577–598.

Lüthje, C. & Franke, N. 2003. The ‘making’ of an entrepreneur: testing a model of entrepreneurial intent among engineering students at MIT. *R&D Management* **33**(2), 135–147.

Maresch, D., Harms, R., Kailer, N. & Wimmer-Wurm, B. 2016. The impact of entrepreneurship education on the entrepreneurial intention of students in science and engineering versus business studies university programs. *Technol. Forecast. Soc. Change* **104**, 172–179.

Martin, B.C., McNally, J.J. & Kay, M. 2013. Examining the formation of human capital in entrepreneurship: A meta-analysis of entrepreneurship education outcomes. *J. Bus. Venturing.* **28** (2), 211–224.

Matlay, H. 2008. The impact of entrepreneurship education on entrepreneurial outcomes. *J. Small. Bus. Ent. Dev.* **15**(2), 382–396.

Matlay, H. & Carey, C. 2007. Entrepreneurship education in the UK: a longitudinal perspective. *J. Small. Bus. Ent. Dev.* **14**(2), 252–263.

Menzies, T.V. &. Paradi, J. C. 2003. Entrepreneurship Education and Engineering Students: Career Path and Business Performance. *Int. J. Enter. Innov.* **4**(2), 121–132.

Mets, T., Kozlinska, I. & Raudsaar, M. 2017. Patterns in entrepreneurial competences as the perceived learning outcomes of entrepreneurship education: The case of Estonian HEIs. *Ind. High. Edu*c. **31**(1), 23–33.

Moberg, S.K., Vestergaard, L., Fayolle, A., Redford, D., Cooney, T., Singer, S., Sailer, K. & Filip, D. 2014. How to Assess and Evaluate the Influence of Entrepreneurship Education: A Report of the ASTEE Project with a User Guide to the Tools. Odense: The Danish Foundation for Entrepreneurship – Young Enterprise.

Morris, M.H., Webb, J.W., Fu, J. & Singhal, S. 2013. A Competency-Based Perspective on Entrepreneurship Education: Conceptual and Empirical Insights. *J. Small. Bus. Manage.* **51**(3), 352–369.

Nabi, G., Liñan, F., Fayolle, A., Krueger, N. & Walmsley, A. 2017. The Impact of Entrepreneurship Education in Higher Education: A Systematic Review and Research Agenda. *Acad. Manag. Learn. Edu.* **16**(2), 277–299.

Neck, H. M. & Corbett, A. C. 2018. The Scholarship of Teaching and Learning Entrepreneurship. *Entrep. Edu. Ped.* **1**(1), 8–41.

Peterman, N.E. & Kennedy, J. 2003. Enterprise education: Influencing students‟ perceptions of entrepreneurship. *Entrep. Theory. Pract.* **28**(2) 129–144.

Pittaway, L. & Cope, J. 2007. Entrepreneurship Education: A Systematic Review of the Evidence. *Int. Small. Bus. J.* **25**(5), 479–510.

Põder, A., Vahejõe, K., Luik, H., Janson, J., Turmann, K. & Pärle, A. 2016. Alumni entrepreneurship survey of Estonian University of Life-Sciences, Estonian Academy of Arts and Estonian Entrepreneurship University of Applied Sciences. Tartu: Estonian University of Life Sciences (in Estonian)

Põder, A., Viira, A.-H. & Värnik, R. 2017. Firm entries and exits in Estonian urban municipalities, urban hinterlands and rural peripheries 2005−2012. *J. Baltic. Stu.* **48**(3), 285−307.

Raudsaar, M. & Kaseorg, M. 2016. Development of Entrepreneurship Education in Estonian Universities: Case of University of Tartu. *The European Proceedings of Social & Behavioural Sciences* (EpSBS) **16**, 828−837.

Reis, D.A., Fleury, A.L. & De Carvalho, M.M. 2019. Contemporary Trends in Engineering Entrepreneurship Education. *Int. J. Eng. Edu.* **35**(3), 824–841.

Souitaris, V., Zerbinati, S. & Al-Laham, A. 2007. Do entrepreneurship programmes raise entrepreneurial intention of science and engineering students? The effect of learning, inspiration and resources. *J. Bus. Venturing.* **22**(4), 566–591.

Täks, M., Tynjälä, P. & Kukemelk, H. 2016. Engineering Students' Conceptions of Entrepreneurial Learning as Part of Their Education. *Eur. J. Eng. Educ.* **41**(1), 53−69.

Täks, M., Tynjälä, P., Toding, M., Kukemelk, H. & Venesaar, U. 2014. Engineering Students' Experiences in Studying Entrepreneurship. *J. Eng. Educ.* **103**(4), 573−598.

Van Praag, C.M. & Versloot, P. 2007. What is the value of entrepreneurship? A review of recent research. *Small. Bus. Econ.* **29**(4), 351–382.

Viira, A-H., Põder, A. & Värnik, R. 2009. 20 years of transition- institutional reforms and the adaptation of production in Estonian agriculture. *Agrarwirtschaft* **58**(7), 286−295.

Agronomy Research **17**(6), 2417–2427, 2019
https://doi.org/10.15159/AR.19.206

Cost efficiency of different cropping systems encompassing the energy crop *Helianthus annuus* L.

E. Skoufogianni, K.D. Giannoulis*, D. Bartzialis and N.G. Danalatos

University of Thessaly, Department of Agriculture, Crop Production & Rural Environment, Fytokoy street, 38443, Volos, Greece
*Correspondence: kyriakos.giannoulis@gmail.com

Abstract. Crop rotation and green manure are the most ancient and popular cropping systems. This study sought to analyze the economic efficiency of sunflower where pea (*Pisum sativum* L.) either harvested or incorporated at the flowering stage in the soil before the sowing of sunflower in Europe and the final agricultural profit of such a cultivation system. Therefore, the main objective of this paper is to report the production costs and to find out which of the tested cultivation system gets sunflower cultivation economically viable in Greece and in Mediterranean region. To assess the economic efficiency, three-year field experiments were established in two contrasting environments in central Greece (Trikala and Larisa) and contained three different cultivation practices using legumes comprised the main-factor (T_1: control, T_2: legume incorporated at the flowering stage, T_3: legume incorporated after seed harvest), while nitrogen fertilization comprised the sub-factor (N_1:0, N_2:50, N_3:100 and N_4:150 kgNha^{-1}). The results derived from this study revealed the positive effect of the legume incorporation treatment (T_2: legume incorporated at the flowering stage) where the final yield increased up to 5 t ha^{-1} regardless region. Moreover, depending on the year the T2 treatment increases the final yield 30–50% and a yield increase was also noticed to the treatment where the legume was harvested (T_3: legume incorporated after seed harvest). Therefore the introduction of this scheme into future land use systems in Greece and more generally in Mediterranean basin should be seriously taken into consideration.

Key words: sunflower**,** pea, monocrop, rotation, green manure.

INTRODUCTION

A challenge for sustainable agriculture is to identify those plants which are most efficient in forming stable soil structure and to incorporate them into economic rotational management systems. Several organic materials have been reported as suitable soil amendments for increasing crop production and the most ancient and famous are crop rotation and green manure (Ruis & Blanco-Canqui, 2017).

It is generally accepted that rotation with cover crops is fundamental for yield increase, as well as for controlling soil erosion and degradation, ground water pollution, and maintaining acceptable organic matter levels in the topsoil (Ruis & Blanco-Canqui, 2017). Soil organic matter represents a major proportion of the organic carbon within a terrestrial biosphere which plays an important role in soil fertility and affects infiltration, water retention, crop growth and productivity (Stępień et al., 2018). As a result, by conserving the soil its yield over time and generally agroecosystem sustainability are ensured (Powlson et al., 2001; Brennan & Smith, 2005; Tonitto et al., 2006; Ordonez et al., 2007; Imogie et al., 2008; Cattanio 2012; Isbell et al., 2017).

Legumes are included in crop systems due to the atmospheric N fixation of nitrogen and are the finest external options that can be used to enhance the efficiency of nitrogen use (Thorup-Kristensen et al., 2003; Fageria & Baligar, 2005; Dorn et al., 2015). *Pisum sativum* has great potential among legume species that could be used as cover crops, as it is well adapted to Mediterranean-type crops with thriving cultivation techniques (Miller et al., 2003). Residue quality is usually associated with two factors: the time to preserve its physical characteristics, and the supply of carbon and mineral elements from its decomposition. These two aspects are affected by both the climate and the structure of the residue (Vergani & Graf, 2015; Bacq-Labreuil et al., 2018) It has been reported (Voroney et al., 1989) that the fixation of organic carbon from pea residuewould sequester 212–318 kg of C ha^{-1}.

Nitrogen (N) is one of the most important elements in the metabolic processes of plants such as protein synthesis and photosynthetic processes. Consequently its use is necessary in order to maximize yields and quality of crops worldwide (Ahmad et al., 2009; Massignam et al., 2009; Ullah et al., 2010).

Nitrogen is a limiting factor that prevents yield in many crops such as sunflower. Sunflower growth depends more on nitrogen than any other nutrient (FAO, 2010). Thus, meeting the needs of the plant in N is necessary for the crop to be profitable (Mortvedt et al., 2003). More specifically the requirements of the sunflower in nitrogen reach 100 kg N ha^{-1} in order to have a good yield (Malligawad et al., 2004). However, nitrogen fertilization is quite variable and depends largely on the soil type and the climate conditions of the area (Chapman et al., 1993; Lauretti et al., 2007). Sunflower has a mean rooting depth of 50 cm that varies with different cultivation managements (e.g. tillage system) (Gajri et al., 1997). Water consumption of sunflower may range from 200 to over 900 mm per season, depending on many factors (like latitude, soil-climate conditions, sowing period and management (Gajri et al., 1997; Debaeke et al., 1998; Karam et al., 2007).

Moreover, it is well documented that sunflower is a crop with low water use efficiency (Rinaldi, 2001; Goskoy et al., 2004), while Mediterranean areas are characterized by mild temperatures and high water availability early in the season, but later on and especially during summer due to greater temperatures and minimum rainfalls, irrigation application seems inevitable (Soriano et al., 2004). In order to reach high seed yields, sunflower should be irrigated at least three times during its growing period, especially during heading, flowering and milking stage, with full or limited irrigation water (Goskoy et al., 2004). Responses to fertilization are also influenced by weather conditions and soil water availability (Lopez-Bellido, 2003). Studies have shown that the optimum amount of N-fertilization is found mainly in the range of 40–190 kg N ha^{-1}, depending on soil type and previous crop (Sirbu et al., 1992; Glas, 1998).

Nitrogen is very essential for plant growth and makes up 1–4% of dry matter of the plants. Moreover, in the case of the comparison between a mono-crop system (industrial crop-industrial crop) and a crop-rotation system (industrial crop - leguminous crop rotation), N application averages 18% and 13% of the variable costs, respectively (Duvick & Cassman, 1999).

There are no data reported about the cost performance of *Pisum sativum*, either harvested or incorporated in the soil before the sowing of sunflower in Europeand the final agricultural profit of such a cultivation system. Therefore, the main objective of this paper is to report the production costs and to find out which of the tested cultivation system makes economically feasible sunflower cultivation in Greece and the Mediterranean region generally.

MATERIALS AND METHODS

Field experiment

Two field experiments were established in two different soils, e.g. in Trikala (West Thessaly; coordinates: 39032 mottle 17.08′ ppl N, 21046 mottle 17.19′ E, 120 m asl)) and in Larisa (East Thessaly; coordinates: 39030 mottle 02.85′ ppl N, 22042 mottle 50.37′ E, 60 m asl), central Greece.

The soil in Larisa (plough layer) is a calcareous (pH: 8.0) clay (sand 2%, silt 35%, clay 63%), fertile (organic matter content 1.7% at 20 cm depth) and was classified as Vertisol (Soil Survey Staff, 1993), while the soil in Trikala area(plough layer) is deep, calcareous (pH: 7.4), sandy clay loam (sand62%, silt 17%, clay 21%), fertile (1.25% organic matter content at 30 cm depth). The soil is characterized as Entisol (Aquic Xerofluvent; Soil Survey Staff, 1993).

For the purposes of the study sunflower (Hybrid *Panter*) was sown using a modern pneumatic seeding machine and the population was 7 plants m^{-2} for both sites. The experimental used design was a factorial split plot with three replications (3 blocks) and 12 plots per block. Three different cultivation practices using legumes (*Pisum sativum* L., cv. caroubi) comprised the main-factor (T_1: control; T_2: legume incorporated at the flowering stage, average 6,000 kg ha^{-1} dry yield; T_3: legume incorporated after seed harvest), while nitrogen fertilization comprised the sub-factor (N_1: 0, N_2: 50, N_3: 100 and N_4: 150 kg N ha^{-1}). The second dose (nitrate form) was applied on the onset vegetative phase of sunflower, at plant height approximately up to 60cm. Sowing dates and other relevant agronomic data are summarized in Table 1. Fertilization for pea was 60 kg Pha^{-1}

Table 1. Agronomic data of pea and sunflower cultivation for both experimental years and location sites

	TRIKALA	
	1st year	2nd year
PEA		
Date of sowing	5/12/2012	1/12/2013
Date of 50% emergence	20/12/2012	27/12/2013
Date of incorporation	13/04/2012	11/4/2013
Average density of plants per m^2	150	150
SUNFLOWER		
Date of sowing	10/06/2012	15/06/2013
Date of 50% emergence	17,19/06/2012	21/06/2013
Date of harvest	1,3/10/2012	1/10/2013
Average density of plants per m^2	20	20
Maximum irrigation applied (mm)	320	350
	LARISA	
PEA		
Date of sowing	5/12/2012	15/11/2013
Date of 50% emergence	20/12/2012	1/12/2013
Date of incorporation	12/04/2012	15/04/2013
Average density of plants	150	150
SUNFLOWER		
Date of sowing	10/06/2012	13/6/2013
Date of 50% emergence	14,19/6/2012	19,20/6/2013
Date of harvest	2/10/2012	1/10/2013
Average density of plants	20	20
Maximum irrigation applied (mm)	320	350

(superphosphate) as basal dressing. The amount of dry biomass of pea incorporated in the top soil was measured directly by means of destructive sampling upon full flowering each year.

During the harvest period (seed maturation) in both areas, a destructive sampling took place by hand, where 1 m^2 in every plot was harvested.

It should be noticed that proper nutrient uptake determinations require at least one extra year of field experimentation, with the plots with same fertilization levels placed exactly at the same position to minimize any residual effect from previous fertilization. This was the case in both field experiments here. The presented data correspond to 2012 and 2013 experimental years, where the crops were sown in the same position for the 2nd and 3rd growing period.

Economic Parameters - Trade off analysis

Besides the agronomic aspects of a cultivation system, analysis of cost–benefit ratio mainly demonstrates farmer's profitability. To assess this, on farm budgets were constructed including three scenarios: i. sunflower as a single crop (mono-crop system), ii. Sunflower as a second crop after legume incorporated at the flowering stage (rotational system), iii. Sunflower as a second crop after legume incorporated after pea seed harvest (rotational system).

The economic costs of the cultivation systems were land rent and establishment costs of each cultivation such as plowing, cheelering, harrowing, sowing, herbicide application, seeds, irrigation, fertilization, incorporation and labor costs for the preparation of the above practices (€ $hour^{-1}$ multiplied with the sum of the needed hours per hectare; existing market prices in Greece were considered), and harvesting costs which depend on the crop (Table 2). Furthermore, to calculate economic returns (per hectare); for the each crop, existing market prices in Greece were considered (the prices refer to the average prices obtained from the survey carried out in the local area during the experimental period). To construct such databases, material and operation costs were considered as well as the local market prices (0.38 and 0.50 € kg^{-1} for sunflower seed and pea grain, respectively).

Table 2. *Pisum sativum* and *Helianthus annuus* cultivation costs in central Greece

€ ha^{-1}	*Pisum sativum incorporated at seed harvest*	*Pisum sativumincorporated at the flowering stage*	*Helianthus annuus*
Culivation costs			
Weed management (labor)	15	15	15
Plowing	90	90	90
Cheeler	40	40	40
Harrowing	60	60	60
Sowing	20	20	30
Seeds	170	170	50
Irrigation	0	0	300
Fertilization	15	15	15
Fertilizer (46-0-0)	150	150	0
Land Rent	700	700	0
Harvest Cost	200	0	150

Statistical analysis

The statistical package GenStat (7th Edition) was used for the analysis of variance (ANOVA) within sample timings for all measured and derived data. The $LSD_{0.05}$ was used as the test criterion for assessing differences between means (Steel & Torrie, 1982).

RESULTS AND DISCUSSION

Sunflower Yield

The positive effect of nitrogen fertilization to the final seed yield of sunflower has been mentioned worldwide (Rinaldi et al., 2001; Zubillaga et al., 2002; Ruffo et al., 2003; Göksoy et al., 2004; Albrizio & Steduto, 2005). The same was also noticed in this study, where the two higher nitrogen fertilization applications (N_3, N_4; 4–4.2 t ha^{-1}) produced 1-1.2 t ha^{-1} higher yield in comparison with the lower levels (N_1, N_2; 3 t ha^{-1}) for both years and areas (Table 3).

Table 3. Sunflower seed yield (kg ha^{-1}) as affected by three cultivation practices and four nitrogen fertilization levels

	Seed Yield Larisa 1st year (kg ha^{-1})	Seed Yield Larisa 2nd year (kg ha^{-1})	Seed Yield Trikala 1st year (kg ha^{-1})	Seed Yield Trikala 2nd year (kg ha^{-1})
T_1	2,537	3,295	3,019	3,895
T_2	4,165	5,197	5,288	5,123
T_3	3,415	4,116	3,844	3,956
$LSD_{0.05}$	*686.0*	*789.7*	*91.4*	*692.7*
N_1	2,826	3,753	3,222	3,283
N_2	3,194	3,887	3,970	3,643
N_3	3,597	4,376	4,432	3,633
N_4	3,663	4,154	4,576	3,784
$LSD_{0,05}$	*209.0*	*103. 8*	*302.5*	*536.4*
CV(%)	*5.5*	*14.5*	*12.3*	*12.0*

*(T_1: control; T_2: legume incorporated at the flowering stage; T_3: legume incorporated after seed harvest; N_1: 0; N_2: 50; N_3: 100 and N_4: 150 kg N ha^{-1}).

The most impressing finding was the positive effect of the legume incorporation treatment (T_2) where the final yield increased up to 5 t ha^{-1} in 2012 for both areas, while in 2013 remained in lower levels 4.72 t ha^{-1}. The higher harvested yield in 2012 was noticed due to higher precipitation during the seed filling stage. Therefore, depending on the year the T_2 treatment will increase the final yield 30–50% which is in agreement with literature (Thorup-Kristensen & Dresboll, 2010). This is due to the nitrogen-binding capacity of the pea, and in particular, during the flowering stage where the nitrogen (N), phosphorus (P) and potassium (K) contents are 10, 3.15, and 3%, respectively, for both sites (data not shown). Finally, it has to be mentioned that there was also found a yield increase to the treatment where the legume was harvested (T_3), which also agree with Stępień et al. (2017) where it is reported an influence of crop rotation on rapeseed seed yield.

It is really important to be mentioned that in sandy soils such as in Trikala, significant yields on seed can be achieved with only small nitrogen applications if the cultivation practice of crop rotation, and in particular that of green fertilization using legumes and in particular peas.

In higher fertility, clay soils such as Larisa, crop rotation has clear benefits in organic carbon and enzyme activities and is related to the percentage of the organic matter as well as the total percentage N. The deep, perennial root system of sunflower utilizes the maximum of the nutrients (Omay et al., 1997; Potter et. al., 1998; Ashraf et al., 2004; Sainju et al., 2006; Lopez-Belido et al., 2010; Melero et al., 2011).

Pea Yield

The blossom stage of pea begins at the Growing Degree Days of 350 °C-d while the seed maturation takes place at 820 °C-d in Larisa and at 947 °C-d in Trikala. The seed growing rate is 100–120 kg ha^{-1} per day, while the final yield reaches the 2,180 kg ha^{-1} in Larisa and the 1,930 kg ha^{-1} in Trikala. The small no significant difference (data not shown) to the final seed yield of the sites is due to the lower soil fertility of the sandy soil in Trikala. Finally, it must be mentioned that pea cultivation reaches the maximum dry yield at the Growing Degree days of 450 °C-d, which is the best period for the incorporation.

Production Costs

The total costs for the different cultivation practices of sunflower using legumes (T_1: control, T_2: legume incorporated at the flowering stage, T_3: legume incorporated after seed harvest) and the different nitrogen fertilization levels (N_1:0, N_2: 50, N_3: 100 and N_4:150 kg N ha^{-1}) are summarized in Fig. 1.

As it was expected, higher average production costs were reported in the case of the T_3N_4 treatment (legume incorporated after seed harvest with 150 kg ha^{-1} nitrogen fertilization), where costs are increased by the fertilization costs and the harvest costs of pea and totally agree with previous findings (Liebman et al., 2012). The required expenses for this case are 1,920 € ha^{-1}. On the other hand, the case with the lower average production costs was the T_1N_1 treatment (control) where the required expenses are 820 € ha^{-1}.

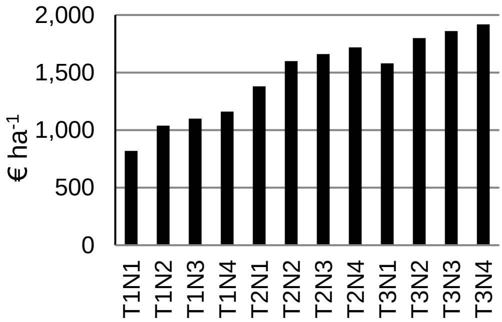


Figure 1. Sunflower cultivation costs as observed in Thessaly plain and as affected by three cultivation practices and four nitrogen fertilization levels. (T_1: control; T_2: legume incorporated at the flowering stage; T_3: legume incorporated after seed harvest; N_1: 0; N_2: 50; N_3: 100 and N_4: 150 kg N ha^{-1}).

Cultivation Profit

Higher profit was found for the treatment where sunflower was harvested as a second crop where pea seed was harvested and the biomass of the legume was incorporated regardless area and year. The average profit for this treatment was 883 € ha^{-1} having a significant difference with the cultivation practice of sunflower as mono-crop system (Table 4).

In the case of the nitrogen fertilization (Fig. 2; Table 4), there was found significant difference for the higher fertilization levels (N_3, N_4) comparing with the lower (N_1, N_2). Moreover, it was found that the cost of the used nitrogen had a negative effect for the treatment with the 150 kg N ha^{-1} (Table 4).

Table 4. Sunflower profit (€ ha^{-1}) as affected by three cultivation practices and four nitrogen fertilization levels

	Profit (€ per ha)				
T_1	143	T_1N_1	225	T_3N_2	858
T_2	456	T_1N_2	53	T_3N_3	945
T_3	883	T_1N_3	137	T_3N_4	907
$LSD_{0.05}$	467.8	T_1N_4	156	$LSD_{0.05}$	379.3
N_1	441	T_2N_1	273		
N_2	429	T_2N_2	376		
N_3	563	T_2N_3	609		
N_4	543	T_2N_4	567		
$LSD_{0.05}$	77.8	T_3N_1	824		
CV (%)	12.1				

*(T_1: control; T_2: legume incorporated at the flowering stage; T_3: legume incorporated after seed harvest; N_1: 0; N_2: 50; N_3: 100 and N_4: 150 kg N ha^{-1}).

As illustrated in Fig. 2, farmers' profit 53 to 225 € ha^{-1} for the single culture of sunflower (cropping scenario monoculture T_1), from 273 to 609 € ha^{-1} and from 824 to 945 € ha^{-1} for the multiple cropping system of sunflower after legume (cropping scenarios T_2, T_3).

Therefore, the above results show a high dependence of sunflower cultivation as a second crop while the extra fertilization of 150 kg N ha^{-1} decreases farmers' profit, with the scenario of incorporation the biomass of pea cultivation after seed harvest and sunflower as second cultivation being more feasible.

Based on the above scenarios, sunflower cultivation as monoculture seems to be not feasible due to the low profit (143 € ha^{-1})if someone includes the fact of the fragmentation of rural land and therefore a farmer needs more than 100 hectares to ensure a sustainable annual income by taking into account the costs of the next year.

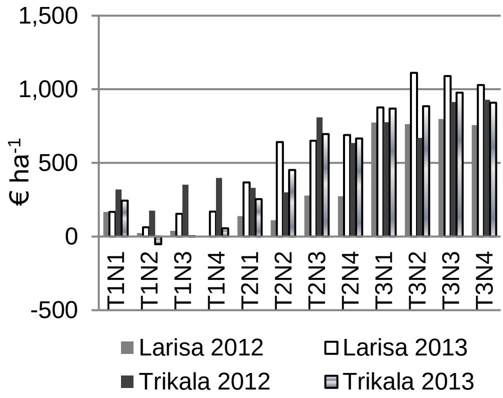


Figure 2. Farmer's profit from sunflower cultivation as observed in Thessaly plain during 2012 and 2013 and as affected by three cultivation practices and four nitrogen fertilization levels. (T_1: control; T_2: legume incorporated at the flowering stage; T_3: legume incorporated after seed harvest; N_1: 0; N_2: 50; N_3: 100 and N_4: 150 kg N ha^{-1}).

Trade off analysis

The economic analysis is for the 2nd and 3rd growing years (2012 and 2013). Finally, sensitivity analysis was used to estimate the effect of product price to crop income benefits for the most viable cultivation system (T_3N_3; 945 € ha^{-1}) estimating that the yield is stable.

Table 5. 2-input table for the T_3N_3 treatment and different seed yield price

Seed Price € per ha	Pea										
Sunflower	0.10	0.15	0.20	0.25	0.30	0.35	0.40	0.45	0.50	0.55	0.60
0.10	-	-	-	-	-	-	-	-	-	-	-
0.15	-	-	-	-	-	-	-	-	-	-	-
0.20	-	-	-	-	-	-	-	-	-	+	+
0.25	-	-	-	-	-	-	-	+	+	+	+
0.30	-	-	-	-	-	+	+	+	+	+	+
0.35	-	-	-	-	+	+	+	+	+	+	+
0.40	-	-	+	+	+	+	+	+	+	+	+
0.45	+	+	+	+	+	+	+	+	+	+	+
0.50	+	+	+	+	+	+	+	+	+	+	+
0.55	+	+	+	+	+	+	+	+	+	+	+
0.60	+	+	+	+	+	+	+	+	+	+	+

*(T_3: legume incorporated after seed harvest; N_3: 100 kg N ha^{-1}).

Therefore, as it shown in Table 5, in case that sunflower seed price is stable at 0.50 € kg^{-1}, the lower price that pea seeds can get is 0.10 € kg^{-1}, while if the seed price of pea is stable the lower price that sunflower seed can get is 0.30 € kg^{-1}. In Table 5 are presented all the price combination for the scenario of pea harvest and the remaining biomass incorporated and the cultivation of sunflower as second crop with nitrogen fertilization of 100 kg ha^{-1} (T_3N_3).

CONCLUSION

Through the different tested cropping systems, single sunflower cropping and multiple cropping of legume followed by sunflower, it was found that the single crop scheme is not economically viable. There was a higher profit for the treatment where sunflower was harvested as a second crop after pea harvest and the remaining biomass is incorporated, irrespective of area and year. The most impressive finding was the positive effect of treatment with legume incorporation with an increase in yield up to 5 t ha^{-1} regardless of region. Depending on the year the incorporation of legume may increases the yield by 30–50 percent while for the treatment where the previous cultivation was legume was also noticed a yield increase.

As a general conclusion, the sunflower cultivation system after pea cultivation appears to be a successful cropping scenario, characterized by high profit for farmers with a possible further rise in profit by enhancing the final yield or raising the seed selling price, and therefore, serious consideration should be given to the implementation of this system into future land use schemes in Greece and, more usually, in the Mediterranean basin.

ACKNOWLEDGEMENTS. The authors acknowledge the Department, Agriculture Crop Production and Rural Environment, University of Thessaly (Greece) for the economic support and access to the literature research database.

REFERENCES

Ahmad, S.R., Ahmad, M.Y. Ashraf, M. Ashraf & Waraich, E.A. 2009. Sunflower (*Helianthus annuus*L.) response to drought stress at germination and seedling growth stages. *Pak. J. Botany* **41**(2), 647–654.

Albrizio, R. & Steduto, P. 2005. Resource use efficiency of field-grown sunflower, sorghum, wheat and chickpea I. Radiation use efficiency. *Agric. & Forest Meteorology* **130**, 254–268.

Ashraf, M., Mahmood, T., Azam, F. & Qureshi, R.M. 2004. Comparative effects of applying leguminous and non-leguminous green manures and inorganic N on biomass yield and nitrogen uptake in flooded rice (*Oryza sativa*L.). *Biol. Fert. Soils* **40**, 147–152.

Bacq-Labreuil, A., Crawford, J., Mooney, S.J., Neal, A.L., Akkari, E., McAuliffe, C. & Ritz, K. 2018. Effects of cropping systems upon the three-dimensional architecture of soil systems are modulated by texture. *Geoderma* **332**, 73–83. doi:10.1016/j.geoderma.2018.07.002

Brennan, E.B. & Smith, R.F. 2005.Winter cover crop growth and weed suppression on the central coast of California. *Weed Technol.* **19**, 1017–1024.

Cattanio, J. 2012. Leaves material decomposition from leguminous trees in an enriched fallow. Federal university of Para-UFPA, Brazil: *INTECH Open* Access Publisher.

Chapman, S.C., Hammer, G.L. & Meinke, H. 1993. A sunflower simulation model. I. Model development. *Agron. J*. **85**, 725–735.

Debaeke, P., Cabelguenne, M., Hillaire, A. & Raffaillac, D. 1998. Crop management systems for rainfed and irrigated sunflower (*Helianthus annuus*) in south-western France. *The Journal of Agricultural Science* **131**, 171–185.

Dorn, B., Jossi, W. & van der Heijden, M.G.A. 2015.Weed suppression by cover crops: comparative on-farm experiments under integrated and organic conservation tillage. *Weed Research* **55**, 586–597.

Duvick, D.N. & Cassman, K.G. 1999. Post-green revolution trends in yield potential of temperate maize in the north-central United States. *Crop Science* **39**, 1622–1630.

Fageria, N. K. & Baligar, V.C. 2005. Enhancing nitrogen use efficiency in crop plants. *Advances in Agronomy* **88**, 97–185.

FAO 2010. Sunflower crude and refined oils. Food and Agriculture Organization of the United Nations. Retrieved from: http://www.responsibleagroinvestment. org /sites/ responsibleagroinvestment.org/files/FAO_Agbiz%20handbook_oilseeds_0.pdf. (Verified June 5, 2013).

Gajri, P.R., Singh, J., Arora, V.K. & Gill, B.S. 1997. Tillage responses of wheat in relation to irrigation regimes and nitrogen rates on analluvial sand in a semi-arid subtropical climate. *Soil Till Res.* **42**, 33–46.

Glas, K. 1998. Sunflower: fertilizing for high yield and quality. *Int. Potash Inst. Bull*. **10**, 1–38.

Goskoy, A.T., Demir, A.O., Turan, Z.M. & Dagustu, N. 2004. Response of Sunflower (*Helianthus annuus* L.) To Full And Limited Irrigation At Different Growth Stages. *Field Crop Research* **87**, 167.

Göskoy, A.T., Demir, A.O., Turan, Z.M. & Dagustu, N. 2004.Responses of sunflower (*Helianthus annuus* L.) to full and limited irrigation at different growth stages. *Field Crops Research* **187**, 167–178.

Imogie, A., Udosen, C., Ugbah, M. & Utulu, S. 2008. Long term effect of Leucanea leucocephala on soil physico-Chemical properties and fresh fruit bunch (FFB). Production of oil palm. *African Journal of plant Science* **2**, 129–132.

Isbell, F., Adler, P.R., Eisenhauer, N., Fornara, D., Kimmel, K. Kremen, C., Letourneau, Deborah K., Liebman, M., H.W. Polley, Quijas, S., Scherer-Lorenzen, M. & Bardgett, R. 2017. Benefits of increasing plant diversity in sustainable agroecosystems. *J. Ecol.* **105**, 871–879.

Karam, F., Lahoud, R., Masaad, R., Kabalan, R., Breidi, J., Chalita, C. & Rouphael, Y. 2007. Evapotranspiration, seed yield and water use efficiency of drip irrigated sunflower under full and deficit irrigation conditions. *Agricultural Water Management* **90**, 213–223.

Lauretti, D., Pieri, S., Vannozzi, G.P., Turi, M. & Giovanardi, R. 2007. Nitrogen fertilization in wet and dry climate. *Helia* **30**(47), 135–140.

Liebman, M., Graef, R.L., Nettleton, D. & Cambardella, C.A. 2012. Use of legume green manures as nitrogen sources for corn production. *Renewable Agriculture and Food Systems* **27**, 180–191.

Lopez-Bellido, R.J., Fontan, J.M., Lopez-Bellido, J. & Lopez-Bellido, L. 2010. Carbon sequestration by tillage, rotation, and nitrogen fertilization in a Mediterranean Vertisol. *Agron. J.* **102**, 310–318.

López-Bellido, R.J., López-Bellido, L., Castillo, J.E. & López-Bellido, F.J. 2003. Nitrogen uptake by sunflower as affected by tillage and soil residual nitrogen in a wheat–sunflower rotation under rainfed Mediterranean conditions. *Soil & Tillage Research* **72**, 43–51.

Malligawad, L.H., Parameshwarappa, K.G. & Giriraj, K. 2004. Studies on the effect of ratios and level of NPK fertilizer nutrients of the productivity of hybrid sunflower under rainfed farming situations. *Proc. 16th Int. Sunflower Conference, Fargo, ND, USA* **1**, 377–386.

Massignam, A.M., Chapman, S.C., Hammer, G.L. & Fukai, S. 2009. Physiological determinants of maize and sunflower achene yield as affected by nitrogen supply. Field Crops Research **113**, 256–267.

Melero, S., Lopez-Bellido, R., Lopez-Bellido, L., Munoz-Romero, V., Moreno, F. & Murillo, J. 2011. Long-term effects of tillage, rotation and nitrogen fertilizer on soil quality in a Mediterranean Vertisol. *Soil & Tillage Research* **114**, 97–107.

Miller, P.R., Gan, Y., McConkey, B.G. & McDonald, C.L. 2003. Pulse crops for the northern Great Plains: II. Cropping sequence effects on cereal, oilseed, and pulse crops. *AgronomyJournal* **95**, 980–986.

Mortvedt, J.J., Johnson, D.L. & Croissant, R.L. 2003. Fertilizing sunflowers. Colorado State Comperative Extension Fact sheet No. 543. *Soil and Crop Sciences* 3/96.

Omay, A.B., Rice, C.W., Maddux, L.D. & Gordon, W.B. 1997. Changes in soil microbial and chemical properties under long-term crop rotation and fertilization. *Soil Sci. Soc. Am. J*. **61**, 1672–1678.

Ordonez-Fernadez, R., Rodriguez-Lizana, A., Carbonell, R., Gonzalez, P. & Perea, F. 2007. Dynamics of residue decomposition in the field in a dryland rotation under Mediterranean climate conditions in southern Spain.*Nutr. Cycl. Agroecosyst.* **79**, 243–253.

Potter, K.N., Tolbert, H.A., Jones, O.R., Matocha, J.E., Morrison, J.E. & Unger, P.W. 1998. Distribution and amount of soil organic C in long-term management system in Texas. *Soil*Till. *Res*. **47**, 309–321.

Powlson, D., Hirsch, P., Brookes, P. 2001. The role of soil microorganisms in soil organic matter conservation in the tropics. *Nutrient cycling in agroecosystems* **61**, 41–51. http://dx.doi.org/10.1007/978-94-015-9008-2-1

Rinaldi, M. 2001. Application of ECIP model for irrigation scheduling of sunflower in southern Italy. *Agric. Water Management* **49**, 185–196.

Ruffo, M.L., Garcia, F.O., Bollero, G.A., Fabrizzi, K. & Ruiz, R.A. 2003. Nitrogen balance approach to sunflower fertilization.*Communication in soil science and plant analysis* **34**(7-18), 2645–2657.

Ruis, S.J. & Blanco-Canqui, H. 2017. Cover crops could offset crop residue removal effects on soil carbon and other properties: a review. *Agron. J*. **109**(5), 1785–1805.

Sainju, U.M., Singh, B.P., Whitehead, W.F. & Wang, S. 2006. Carbon supply and storage in tilled and non-tilled soils as influenced by cover crops and nitrogen fertilization. *J. Environ. Qual*. **35**, 1507–1517.

Sirbu, M. & Ailincai, D. 1992. The effects of long term fertilizer applications including NP on the grain yield and quality of sunflowers. *Cerci Agron*. Moldova **25**, 81–187.

Soil Survey Staff. 1993. Soil survey manual. Soil Conservation Service. U.S. *Department of Agriculture Handbook* 18.

Soriano, M.A, Orgaz, F, Villalobos, F.J. & Fereres, E. 2004. Efficiency of water use of early plantings of sunflower. *Eur. J.Agron*. **21**, 465–476.

Steel, R.G.D. & Torrie, J.H. 1982. Principles and Procedures of Statistics. A Biometrical Approach, 2nd ed., *McGraw-Hill*, Inc., pp. 633.

Stępień, A., Wojtkowiak, K. & Pietrzak-Fiećko, R. 2018. Influence of a crop rotation system and agrotechnology level on the yielding and seed quality of winter rapeseed (*Brassica napus* L.) varieties Castille and Nelson. *J. Elem.* **23**(4), 1281–1293. doi: 10.5601/jelem.2017.22.4.1558

Thorup-Kristensen, K. & Dresboll, D.B. 2010. Incorporation time of nitrogen catch crops influences the N effect for the succeeding crop. *Soil Use and Management* **26**, 27–35.
Thorup-Kristensen, K., Magid, J. & Jensen, L.S. 2003. Catch crops and green manures as biological tools in nitrogen management in temperate zones. *Advances in Agronomy* **79**, 227–302.
Tonitto, C., David, M.B. & Drinkwater, L.E. 2006. Replacing bare fallows with cover crops in fertilizer-intensive cropping systems: A meta-analysis of crop yield and N dynamics. *Agric. Ecosyst. Environ.* **112**, 58–72.
Ullah, M.A., Anwar, M. & Rana, A.S. 2010. Effect of nitrogen fertilization and harvesting intervals on the yield and forage quality of elephant grass (*Pennisetum purpureum*) under mesic climate of Pothowar plateau. *Pak. J. Agri. Sci.* **47**, 231–234.
Vergani, C. & Graf, F.2015. Soil permeability, aggregate stability and root growth: a pot experiment from a soil bioengineering perspective. *Ecohydrology* **9**, 830–842.
Voroney, P., Paul, E. & Anderson, D.W. 1989. Decomposition of straw and stabilization of microbial products. *Canadian Journal of Soil Science* **69**(1), 63–77.
Zubillaga, M.M., Aristi, J.P. & Lavado, R.S. 2002. Effect of phosphorus and nitrogen fertilization on sunflower (*Helianthus annuus* L.) nitrogen uptake and yield. *J. Agronomy & Crop Science* **188**, 267–274.

Agronomy Research **17**(6), 2428–2437, 2019
https://doi.org/10.15159/AR.19.220

Floor temperature as a risk factor for the quality of the environment in the chickens

V. Šranková, J. Lendelová*, M. Žitňák, I. Karandušovská, T. Szabóová, Š. Mihina, M. Balková and Š. Pogran

Slovak University of Agriculture in Nitra, Faculty of Engineering, Department of Building Equipment and Technology Safety, Tr. A. Hlinku 2, SK949 76 Nitra, Slovakia
*Correspondence: jlendelovauniag@gmail.com

Abstract. The aim of the work is to analyze the environmental risk factors in chicken breeding in relation to the heat load of animals in summer. The research was carried out at a breeding hall with a capacity of 20,000 ROSS 308 broiler chickens for two summer period, with a breeding time of 39 days each. The indoor air temperature and relative humidity were continuously measured at two locations at a height of 0.8 m above the floor, using PT 100 temperature sensors and RS 800 humidity sensors. Data were recorded via a PLC unit at 10-minute intervals. The surface temperatures of the floors were measured by DS 80 sensors connected to dataloggers in two locations. It was observed that the floor temperature had an increasing tendency – due to heating, heat produced by animals and anaerobic biological processes – even during the second half of the breeding period. During the period from day 26 to day 39, the surface temperature of the concrete floor, as well as the temperature of the straw bedding rise to above 30 °C. The indoor air temperature in the hall was predominantly decreasing from the 26th day with rising floor temperature tendency. Between the day 26 and day 39 of the breeding period, the average litter temperature elevation over the air temperature exceeded 7 °C. Regression analysis showed negative dependence of floor temperatures on air temperature; for a 1 K unit air temperature reduction, an average floor temperature increase of 0.75 and 1.16 K was found, respectively.

Key words: floor construction, microclimatic parameters, summer period, heat load of chickens.

INTRODUCTION

Despite the many challenges faced by farmers, including environmental problems, diseases, economic pressure and feed availability, climate factors are among the primary and decisive limiting factors for animal development in the warm parts of the world (Renaudaeau et al., 2012). Rising fear of production losses due to high ambient temperatures is justified not only for the tropical areas, but also for countries in temperate zone, where thermal stress is an occasional problem during the 2–3 summer months (Nienaber & Hahn, 2007).

In extreme climatic conditions during the summer season, heat stress can cause high animal mortality, leading to economic losses (Bustamante et al., 2012). Broiler breeding is mainly carried out on litter, the condition of which can be affected by the type of material used, type of flooring under bedding, depth of the material, floor area per animal, feed composition, ventilation system and season.

If indoor environment quality and litter quality are not optimal, there is a significant risk of developing respiratory diseases or dermatitis (Cengiz et al., 2013; De Jong et al., 2014). Properly chosen bedding material provides the animals with greater comfort, serves as a good insulator in winter, operates as absorbent and contaminant modifier (Sigroha et al., 2017). Optimal depth of litter, which contributes to environmental hygiene, leg health, and animal stress reduction, is also important, as is the minimalization of insect-related problems. Bedding materials as wheat straw, sawdust or shavings are usually chosen according to their local availability. In conditions of Slovakia, wheat straw (almost 85%) is the most widely used. Floor and bedding directly affect animal welfare. Special attention must be paid to the litter in order to maintain the thermal balance between the chicken body and the environment. The litter temperature is variable (Nawalany, 2012; Boďo & Gálik, 2018) but should be similar to the air temperature in the housing (Fiorentin, 2006).

The aim of this work was to analyze environmental risk factors in broiler breeding in relation to the animal heat load in summer. An investigation was undertaken to observe whether the following indoor conditions were met:

– the average daily indoor air temperature in an insulated breeding hall, evaluated during the whole summer fattening period being within the recommended temperature range for the given breed of chickens,

– the litter temperature and the temperature of concrete under the bedding being equal to or lower than the air temperature during summer,

– the surface temperature of the floor is changing in dependence on the air temperature in the breeding environment during entire period.

MATERIALS AND METHODS

The measurements were carried out in a breeding hall with a capacity of 20,000 ROSS 308 broiler chickens for two summer breeding periods, each with a breeding time of 39 days. The chickens were housed on a deep straw litter with thickness of 100 mm. The total length of the hall is 104 m, with a width of 12.8 m. The breeding area for chickens is 12,684.1 m^2. To decrease heat load of animal there is 13.3 broiler chickens for m^2 in summer time, because initial number of chickens is decreasing to 17,000.

The external walls of the hall consist of two 7 mm thick Ezalit boards, between which polystyrene foam insulation with thickness of 50 mm is input. The roof structure too, consists of a sandwich made of Ezalit, polystyrene, Ezalit and sheet metal cover. The floor consists of 20 mm thick cement screed, 150 mm thick concrete screed, 100 mm thick slag and 150 mm thick gravel backfill.

Thermal insulation of the hall is represented by thermal resistances R in $m^2\ K\ W^{-1}$. It was calculated according national standard STN 73 0540 (as the quotient of the material thickness ‘d’ in meter and the thermal conductivity coefficient ‘λ’ in $W\ m^{-1}\ K^{-1}$ for all particular structural layers): with results for walls $R_w = 1.37\ m^2\ K\ W^{-1}$, for ceiling $R_c = 1.42\ m^2\ K\ W^{-1}$, for floor $R_f = 1.72\ m^2\ K\ W^{-1}$. Air exchange process is ensured by five ceiling fans with an output of 13,800 $m^3\ h^{-1}$, three front fans with a capacity of 36,000 $m^3\ h^{-1}$ and 44 suction air flaps with dimensions of 500 mm x 200 mm located on both side walls (Fig. 1).

Ventilation is controlled automatically on the basis of climate sensors located in the center of the hall. The heating of the hall is provided by four ERMAF GP 70 gas heaters with power of 70 kW. Microclimatic parameters are regulated according to the values recommended by ROSS (2018).

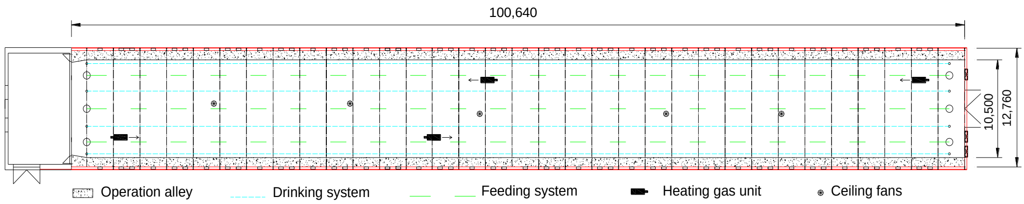


Figure 1. Scheme of chicken house.

The recommended indoor air temperature is defined by an appropriate range of the most frequent relative humidity range of 40% to 70%, with the relative humidity range from 60% to 70% considered as ideal.

Development of interior temperature is controlled in time sequence from the day 1 to day 27 of the chicken age according to the relative air humidity. Accordingly, the desired temperature range varies from 29.2 °C–36 °C on the 1st day of the chicken age to 19.3 °C–24.8 °C on day 27 of the chicken age. After day 27, the temperature usually should have a steady trend.

The temperature and relative humidity of the outside air were measured during the entire period using the COMET S3121 datalogger with a recording rate of 10 minutes, located near the experimental hall at a distance of 50 m. The indoor air temperature and relative humidity were continuously measured at two locations at a height of 0.8 m above the floor using PT 100 temperature sensors (T1, T2) and RS 800 humidity sensors (RH1and RH2) (Fig. 2). Data were recorded via a PLC collection unit at 10-minute intervals. The surface temperatures of floors were measured by DS 80 sensors connected to data loggers at two locations (Fig. 3).

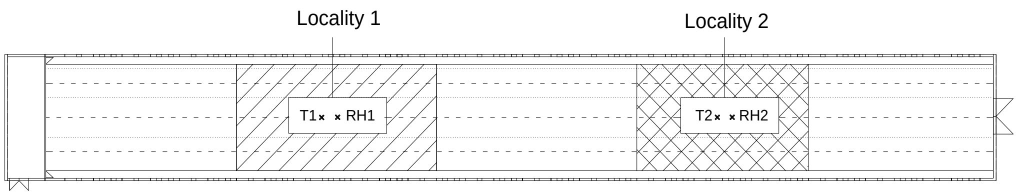


Figure 2. Measurement points of climatic parameters in the experimental hall (T1, T2 – points of measurement of air temperature; RH1 and RH2 – points of measurement of relative air humidity).

The fattening period was divided into three phases: phase P1 (from day 1 to day 13 of the period), phase P2 (from day 14 to day 26 of the period), phase P3 (from day 27 to day 39 of fattening period).

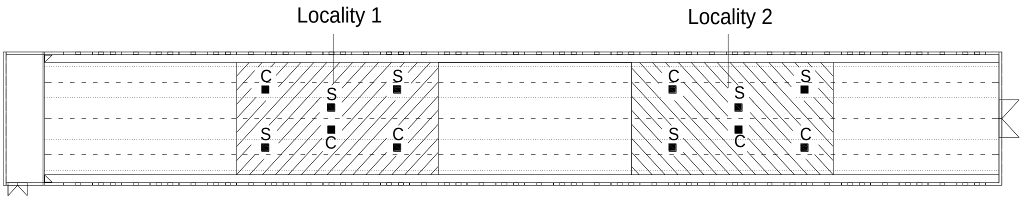


Figure 3. Measurement points of floor surface temperatures in locality 1 and locality 2 (S – straw bed temperature sensors; C – concrete floor surface temperature sensors).

Mortality assessments were performed on the basis of data on daily mortality based on the time dependence of mortality development from the first to the last fattening day. There were evaluated only summer periods where no diseases were recorded, no mistakes were detected in ventilation (with permanently air exchange of 177,000 $m^3 h^{-1}$) and deaths were connected only with hot wave during last phase of breeding period.

Evaluation of the measured data was performed using the 'STATISTICA 10'. By means of a single-factor analysis of ANOVA and the Scheffe test, the measured air and floor temperatures were compared at a significance level of 0.05.

A regression analysis was used to evaluate the floor temperature vs. air temperature, for which the data from the last phase P3 were used.

RESULTS AND DISCUSSION

In phase P3 of both L1 and L2 periods, measured air temperature exceeded the required temperature (ROSS limits, 2018) by 0.9 °C to 8.4 °C for the current relative humidity level. At the same time, it was found that the mortality of animals was demonstrably higher than in the previous phases ($P < 0.05$). No bacteriological infections or diseases were identified in either period, it can thus be believed that the increased mortality was caused by unbearable heat load. In the L1 turnover, the average daily RH ranged from 42.4% to 66.5% with the occurrence of hourly maximum values of up to 79.7%. In the second summer L2 period, RH ranged from 51.5 to 65.8%, with an hourly maximum of 82.4%. However, days with over limit temperatures or relative humidity above 70% were not regularly linked with increased death.

From the results of the measurement of the floor surface temperatures, it was found that the floor constructions temperature has a permanently increasing tendency, due to heating and heat produced by the animals (Figs 4 and 5). During the second third (from day 18 of the period) the surface temperature of the floor constructions also exceeded the required air temperature limits and contributed to the increase in the ambient temperature. The surface temperature of the concrete floor ($T_{f,c}$), as well as the temperature of the straw bedding ($T_{f,b}$), with which the animals are in direct contact while resting, gradually rise to values exceeding 33 °C. The indoor air temperature in the hall had a predominantly decreasing tendency as the floor temperature increased.

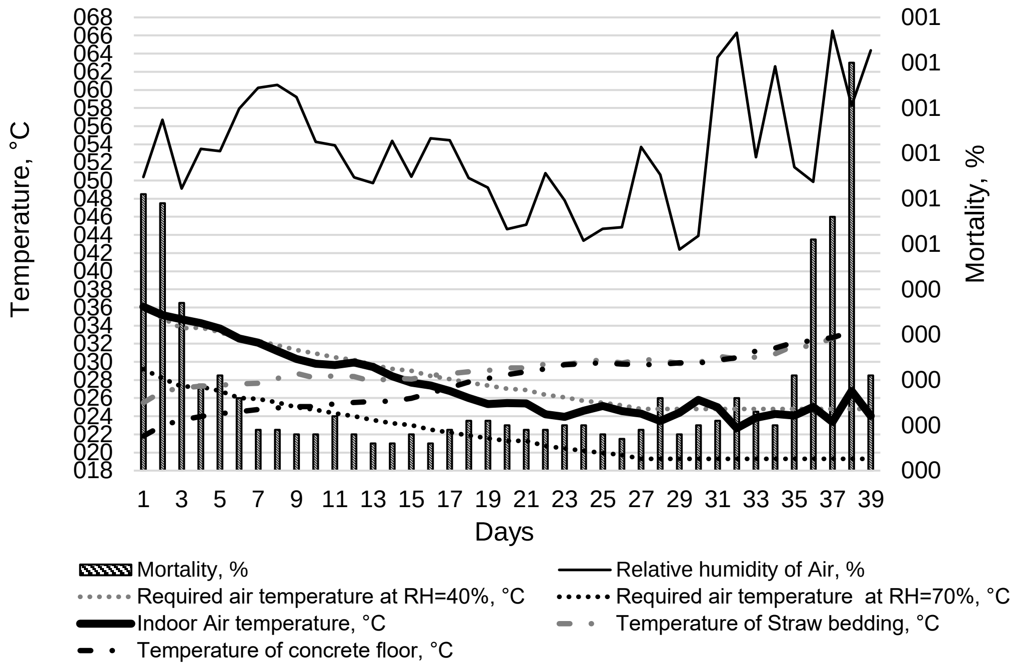


Figure 4. Microclimate parameters and floor surface temperatures and mortality during the L1 period.

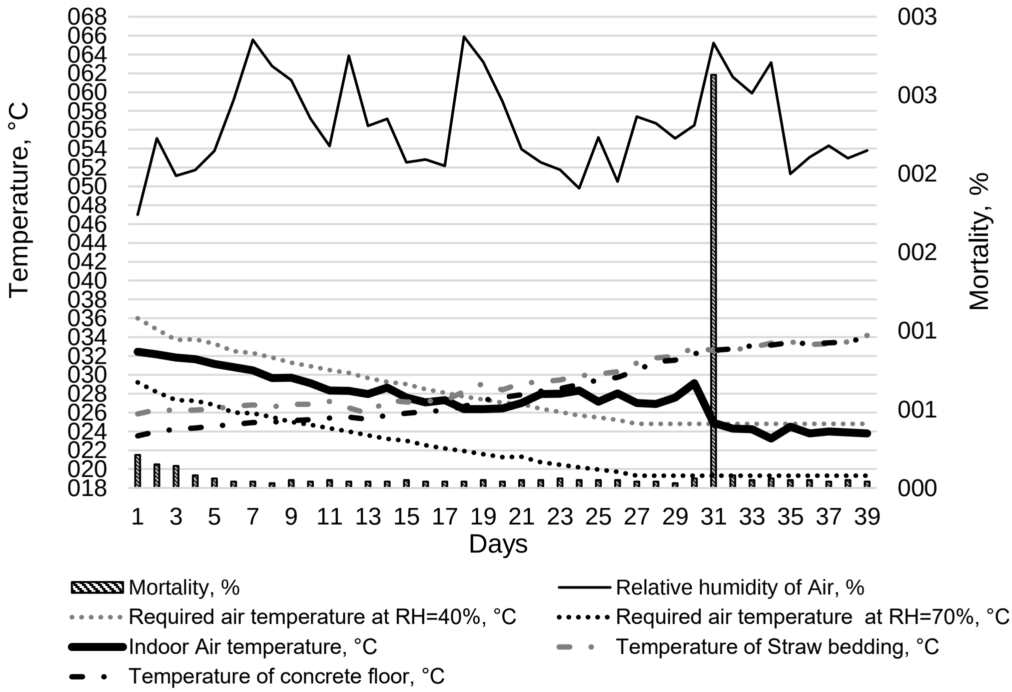


Figure 5. Microclimate parameters and floor surface temperatures and mortality during the L2 period.

The average temperature of 100 mm straw bedding increased from $T_{f,b,d1,L1}$ = 26.29 °C to $T_{f,b,d39,L1}$ = 33.20 °C in period L1 (similar in period L2 from $T_{f,b,d1,L2}$ = 25.49 °C, to $T_{f,b,d39,L2}$ = 33.20 °C). The average surface temperature of the concrete floor under the bedding also increased throughout the entire period although at the start of the period it was around 3 °C lower than the bedding temperature. However, the temperatures of straw and concrete gradually equalized. The continuously increasing temperature difference between air and straw reached $\Delta T_{d39,L1}$ = 8.7 K and $\Delta T_{d39,L2}$ = 10.32 K by the end of the breeding period. Based on the Scheffe contrast test, the average air temperature $T_{a,L1}$ = 24.42 °C in the last period P3 (from day 27 to day 39) was shown to be significantly lower ($P < 0.05$) than the average concrete temperature $T_{f,c,L1}$ = 31.27 °C and straw bedding temperature $T_{f,b,L1}$ = 31.15 °C. A similar trend of temperature development in cases of concrete floor and straw bedding was also observed in the second summer period L2 (Fig. 7). In monitoring the dependence of the floor temperature development on air temperature, a negative interdependence was found: in the L1 period, with a unit air temperature decrease of 1K, an average increase of 0.75 K Was observed; in the L2 an average increase of 1.16 K Was found (Figs 8 and 9).

During L1 and L2 period, a significant difference ($P < 0.05$) was observed between the periods considered, where the average total broiler mortality in the last third of the period (P3) was significantly higher than the mortality in P1 and P2 (Fig. 6).

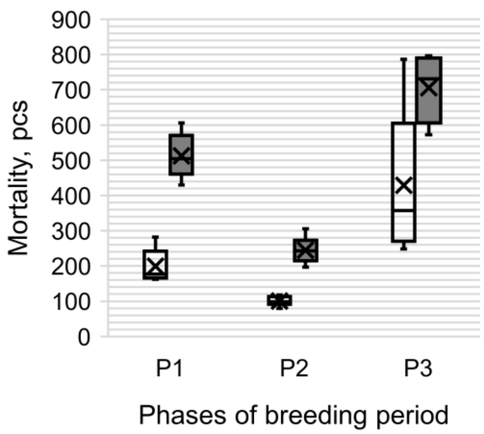


Figure 6. Broiler mortality of all 6 halls in phase P1 (d_1–d_{13}), phase P2 (d_{14}–d_{26}), phase P3 (d_{27}–d_{39}) for fattening period L1 (white boxes) and period L2 (gray boxes); differences between mortality in phase P3 and phase P2 were significant ($p < 0.05$) in both summer periods.

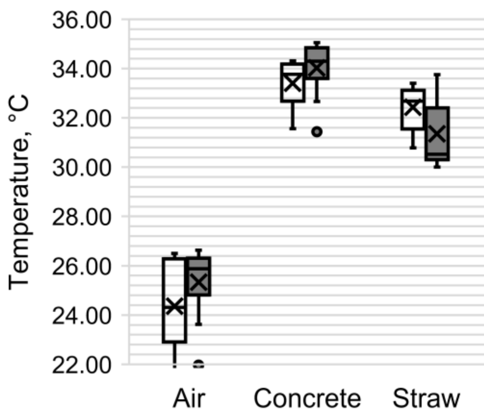


Figure 7. Results of air temperature and floor temperature with F-test during phase P3 in period L1 (white boxes) and L2 (gray boxes); differences between air temperatures and floor temperatures were significant ($p < 0.05$) in both summer periods.

Animal welfare was observed in both monitored periods. In the first half of the breeding, the ambient temperature was in the range of recommended values. However, due to the outside weather, the stabilization period after the 27th day was not without problems, due to the influx of hot waves, which were the most hazardous during the last phase P3 of period L1 and L2, too.

In accordance with the ROSS Industrial Guideline (2018), litter with a temperature of 28 °C–30 °C is emphasized in the early days of chicken age.

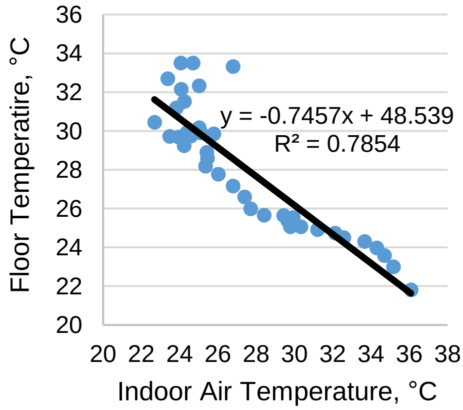


Figure 8. Negative dependence between indoor air temperature and floor temperature in breeding summer period L1 ($R^2 = 0.7854$).

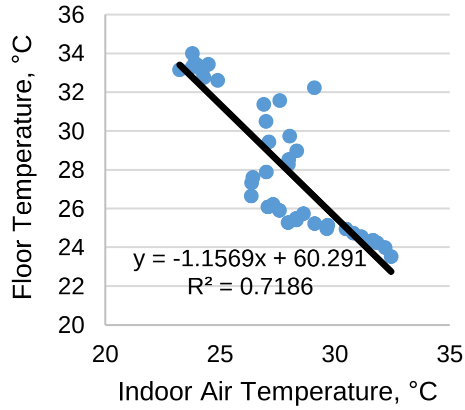


Figure 9. Negative dependence between floor temperature and air temperature in breeding summer period L2 ($R^2 = 0.7186$).

Conditions of breeding environment with overly high temperatures are harmful according to Oliviera et al. (2006), as with age, chickens become more sensitive to high ambient temperatures (Moreira et al., 2004; Nascimento et al., 2018), which results in reduced feed intake, heat dissipation and animal welfare. In industrial poultry farming, the maintenance of homeothermia must be ensured while maintaining animal welfare and minimum production and energy costs (Cangar et al., 2008, Santos et al., 2009). The ability of the birds to dissipate heat is reduced when the ambient temperature and relative humidity increase above the thermoneutral zone (T_{ai} = 24 °C, RH_{ai} = 70%). During the measurements carried out in the summer fattening period L1, an average daily temperature $T_{a,i,30d}$ = 25.1 °C and $RH_{a,i,30d}$ = 63.57% was found, which appeared to be in the tolerable zone of thermal comfort. However, the observed animal deaths pointed to critical circumstances, which were not detected until a detailed analysis of the temperatures at smaller hourly intervals. Increased mortality occurred in the final phase of the fattening period L2, when indoor air temperature exceeded 24 °C for 5 hours or longer and relative indoor air humidity of over 70% lasted for more than 15 hours. The statement by Nascimento (2011) that the surface temperature of floors in the broiler breeding area varies depending on the temperature of air in the breeding environment and is not affected by the age of the broilers is noteworthy in the given context. Some deviations from this statement were noted in the performed experiments. First, the temperature of the floor increased with the age of the chickens, in the last phase of the fattening period even exceeding the air temperature by an average of 6.75 °C during L1 and 8.52 °C during fattening period L2. It can be assumed that the floor was heated not only by air, but also by the litter fermentation processes and the body heat of the growing chickens. Chepete et al. (2005) and Jones et al. (2005) demonstrated the importance of average daily temperature in predicting increased broiler mortality resulting from thermal stress. The average daily air temperatures exceeded the recommended values for critical RH = 40% on days 29, 30, 35 and 37 in the observed fattening period L1 (gray dashed line of desired temperatures in Fig. 3). Elevated temperatures during the first two days (day 29 and day 30) did not cause increased deaths, but humidity remained within acceptable limits and animal weight, and age was lower than for the second two days (day 35 and day 37) when the relative humidity increased with age and animal weights. Then, extremely high mortality occurred on days 36, 37, and 38 of the fattening period. The average daily outdoor air temperature varied from the minimum temperatures of $T_{a,o,min,L1}$ = 14.30 °C to the maximum temperatures $T_{a,o,max,L1}$ = 27.9 °C. Similar findings were made on the L2 period, when air temperature due to high relative humidity on day 29 resulted in increased mortality the following day M_{d31} = 428 animals. The litter temperature should be approximately the same as the air temperature in the housing area (Fiorentin, 2006). In the observed period, the litter temperature at the start of the fattening was 6.54 °C to 9.71 °C lower than the air temperature. However, at the end of the fattening period, it was 7.12 °C to 10.38 °C higher than the air temperature. Abreu et al. (2011) also reported that the temperature was equal to the indoor air temperature after the day 18 of breeding and continued to rise to 30 °C–34 °C over the following days. The litter temperature was slightly reduced or constant by the end of fattening. This trend has been confirmed by the performed measurements, as the litter temperature has been increasing tendency. Increased litter temperature under the sitting chickens is not a necessary result but is related to the fact that litter is usually a good insulator that prevents heat dissipation from the animal's body during sitting. The contact temperature may approach the core temperature of 41.11 °C if the litter produces more heat (Czarick et al., 2016). May & Lott (2000) consider mortality to be the best indicator of environmental temperature impact on efficacy and observed parameters of broiler chicken breeding quality. Heat stress can lead to mass mortality in birds and big losses for farmers over a short time period (Zou, 2014). Measures resulting from multi-annual research monitoring of animal mortality in relation to temperature-moisture balance in real objects will have to be applied very soon in pracitce.

Generally, cooling is achieved by means of rapid removal of heat by high speed air flow ($v > 2.0$ m s^{-1}), any of the evaporative cooling systems (adiabatic coolers, PAD systems, sprayers, etc.), or system combinations. High velocity air flow achieves a significant effect in tunnel ventilation, especially in the final phase of breeding, which compensates for investment costs. Another method of cooling is the combination of underfloor heating and cooling, developed by Nawalany et al. (2010). As early as during entry experiments, they showed that mortality in the 5th and 6th week of breeding is reduced by 50% compared to the traditional method of breeding on the straw.

CONCLUSIONS

The results of the measurements carried out in the chicken fattening hall during the two summer fattening periods can be summarized as follows:

– The average indoor air temperatures obtained from the 24–hour daily measurements over the entire fattening period in the evaluated heat-insulated hall exceeded the recommended temperature ranges for 4 days.

– The continuously measured surface temperature of litter and concrete under the litter in the hall was higher than indoor air temperature during the second half of fattening period, with a predominantly increasing trend; the excess of the litter temperature compared to the air temperature was more than 7 °C even in the third period (from day 27 to day 39).

– Regression analysis has shown a negative dependence of litter and concrete temperature on air temperature; for a 1 K air temperature reduction, an average floor temperature increase of 0.75–1.16 K Was observed.

In breeding practice, on the basis of measurements of climatic parameters and surface temperatures in the thermally insulated hall, other ways of increased animal heat load solutions in summer are profiled in addition to the evaporative cooling solution and tunnel ventilation.

Since it has been found that by the end of period the highest temperature increase is concentrated in the floor and above the floor, it is advisable to install additional methods of animal cooling directly in this area.

Based on practical measurement experience, it is possible to recommend installation of additional evaporation units operating in the transverse direction, followed by directing the developed cooled air into the animal zone, as well as the use of heat pumps and other renewable energy sources.

ACKNOWLEDGEMENTS. This research was supported by project NFP 26220220014 and knowledges gained from LivAGE (project COST): Ammonia and Greenhouse Gases Emissions from Animal Production Buildings was used.

REFERENCES

Abreu, V.M.N., Abreu, P.G., Jaenisch, F.R.F., Coldebella, A. & De Paiva, D.P. 2011. Effect of floor type (Dirt or Concrete) on litter quality. *Brazilian Journal of Poultry Science* **13**(2), 127–137.

Boďo, Š. & Gálik, R. 2018. Monitoring of litter quality and temperature in breeding of fattening chickens. In *Acta Technologica Agriculturae* **21**(1), 33–37.

Bustamante, E., Guijarro, E., García, F.J., Balasch, S., Hospitaler, A. & Torres, A.G. 2012. Multisensor system for isotemporal measurements to assess indoor climatic conditions in poultry farms. *Sensors* **12**(5), 5752–5754.

Cangar, Ö., Aerts, J., Buyse, M.J. & Berckmans, D. 2008. Quantification of the spatial distribution of surface temperatures of broilers. *Poultry Science* **87**, 2493–2499.

Cengiz, O., Hess, J.B. & Bilgili, S.F. 2013. Effect of protein source on the development of footpad dermatitis in broiler chickens reared on different flooring types. *European Poultry Science* **77**(3), 166–170.

Chepete, H.J., CHimbombi, E. & Tsheko, R. 2005. Production performance and temperature-humidity index of COBB 500 broilers reared in open-sided naturally ventilated houses in Botswana. *American Society of Agricultural Engineers* **7**, 524–535.

Czarick, M., Hicks, M. & Fairchild, B. 2016. Are birds cooler at night when the lights go out? *Poultry Housing Tips* **28**(3), 1–6.

De Jong, I.C., Gunnink, H. & Hindle, V. 2014. *Implementation of the Welfare Quality broiler monitor on Dutch broiler farms.* Wageningen UR Livestock Research, Wageningen, pp. 56.

Fioretin, L.A. 2006. Reutilization of aviary bed in the context of benchmarking. *Avicultura Industrial* **97**(6), 12–18.

Jones, T.A., Donnelly, C.A. & Stamp, D.M. 2005. Environmental and management factors affecting the welfare of chickens on commercial farms in the United Kingdom and Denmark stocked at five densities. *Poultry Science* **84**(2), 1155–1165.

May, J.D. & Lott, B.D. 2000. The effect of environmental temperature on growth and feed conversion of broilers to 21 days of age. *Poultry Science* [online] **79**(1), 669–671.

Moreira, J., Mendes, A.A., Roça, R.O., Garcia, E.A., Naas, I.A., Garcia, R.G. & Paz, I.C.L.A. 2004. Effect of stocking density on performance, carcass yield and meat quality in broilers of different commercial strains. *Brazilian Journal of Animal Science* **33**(6), 1506–1519.

Nascimento, D.C.N., Dourado, L.R.B., Siqueira, J.C.D., De Lima, S.B.P., Da Silva, M.C.M., Da Silva, G.G., Sakomura, N.K., Ferreira, G.J.B.C. & Biagiotti, D. 2018. Productive features of broiler chickens in hot weather: effects of strain and sex. *Ciencias Agrárias* **39**(2), 731–746.

Nascimento, G.R., Nääs, I.A., Pereira, D.F., Baracho, M.S. & Garcia, R. 2011. Assessment of broiler surface temperature variation when exposed to different air temperatures. *Brazilian Journal of Poultry Science* **13**(4), 259–263.

Nawalany, G., Bieda, W. & Radon, J. 2010. Effect of floor heating and cooling of bedding on thermal conditions in the living area of broiler chickens. *European Poultry Science* **74**(2), 98–101.

Nawalany, G. 2012. A proposal to apply operative temperature for the evaluation of thermal conditions in the broiler living zone. In *Archiv fur Geflugelkunde* **76**(1), 49–54.

Nienaber, J.A. & Hahn, G.L. 2007. Livestock production system management responses to thermal challenges. *International Journal of Biometrology* **52**(2), 149–157.

Oliveira, R.F.M., Donzele, J.L., Abreu, M.L.T., Ferreira, R.T., Vaz, R.G.M.V. & Cella, P.S. 2006. Effects of temperature and relative humidity on performance and yield of noble cuts of broilers from 1 to 49 days old. *Brazilian Journal of Animal Science* **35**(3), 797–803.

Renaudeau, D., Collin, A., Yahav, S., Baselio, V., Gourdine, J.L. & Collier, R.J. 2012. Adaptation to hot climate and strategies to alleviate heat stress in livestock production. *Animal* **6**(5), 707–728.

ROSS. 2018. *Ross broiler management handbook*. Aviagen, 148 pp. Available at: http://en.aviagen.com/assets/Tech_Center/Ross_Broiler/Ross-BroilerHandbook2018-EN.pdf

Santos, P.A., Baêta, F.C., Tinôco, I.F.F., Albino, L.F.T. & Cecon, P.R. 2009. Ventilação em modos túnel e lateral em galpões avícolas e seus efeitos no conforto térmico, na qualidade do ar e no desempenho das aves. *Revista Ceres* **5**6(2), 172–180.

Sigroha, R., Bidhan, D.S., Yadav, CH.D., Sihag, S.S. & Malik, A.K. 2017. Effect of different litter materials on the performance of broiler chicken. *Journal of Animal Research* **7**(4), 665–671.

Šranková, V., Lendelová, J., Mihina, Š., Žitňák, M. & Nawalany, G. 2019. Mortality of broiler chickens during summer fattening periods affected by microclimatic conditions. In *Acta technologica agriculturae* **22**(1) 22–30.

STN 73 0540-2+Z1+Z2. 2019. Thermal protection of buildings. Thermal performance of buildings and components. Part 2: Functional requirements. Consolidates version.

Zou, Y. & Hrnjak, P.S. 2014. Effect of oil on R134a distribution in the microchannel heat exchanger with vertical header. *International Journal of Refrigeration* **40**, 201–210.

Agronomy Research **17**(6), 2438–2444, 2019
https://doi.org/10.15159/AR.19.215

Change of physical properties of arable chernozem in the initial period of the after agricultural abandonment regime

A.V. Trushkov*, M.Y. Odabashyan, K.Sh. Kazeev and S.I. Kolesnikov

Southern Federal University, Academy of Biology and Biotechnology named after D.I. Ivanovsky, Stachki Ave. 194/1, RU344090 Rostov-on-Don, Russia
*For correspondence: trushkov_tolik@mail.ru

Abstract. A field experiment was conducted in the botanical garden of the South Federal University (Rostov-on-Don, Russia), which was aimed at converting the old arable land plot to the arable regime. Physical properties of chernozems were studied during the first years of the postagrogenic period in different plots, such as: a virgin steppe plot, an arable plot and an abandoned plot. During the course of the experiment it was revealed that physical properties of postagrogenic soils change due to vegetation development after tillage is discontinued. Within three years of research a biological diversity of the floristic composition in the abandoned plot increased from 9 species (during the first year) up to 38 species of plants (3 years later). Vegetation development served as a cause of changes in physical properties of chernozems. Temperature of the abandoned soils decreased along with soil moisture growth, if compared to the relevant indices of the arable plot of land. Owing to the root development and cessation of the agricultural impact, density of the upper horizon in the abandoned plot dropped by 10% on average. A positive correlation was revealed between the chernozem density and its penetration resistance ($r = 0.70$) and temperature ($r = 0.73$), whereas an inverse correlation was detected between the chernozem density and its moisture content ($r = -1.0$).

Key words: agrogenic impact, demutation, succession, soil density, soil penetration resistance, hydrothermal conditions.

INTRODUCTION

The official census of the land cadaster in Russia, conducted in 2016, revealed 44% of abandoned land plots, being unused in agriculture. Constant climate change and human anthropogenic activities reduce territories suitable for arable farming every day. Over 34% of the world's arable lands are more or less exposed to degradation (Kudeyarov, 2019). Large territories of the steppe land are transformed by people, with that entailing destruction of unique plants, depletion of the wildlife and changes in the soil cover.

Taking into account anthropogenic load on soils, physical properties of soil are an important factor that determines soil fertility (Kalinina et al., 2015; Gorbov et al., 2016). Soil fertility depends upon a constant need of plants in nutrients, optimal moisture, temperature and air access required for normal life of microorganisms. Hydrothermal regime of soil is an important factor of its fertility that determines development of various processes. Soil temperature along with its moisture content has a major impact upon the main soil-formation processes, biomass accumulation and biological productivity (Kechaikina, 2011; Myasnikova et al., 2013). Succession processes taking place in abandoned soils as a result of the abandoned land overgrowing with vegetation fall into the secondary postagrogenic successions category (reconstructive successions) (Kazantseva et al., 2008; Myasnikova et al., 2013). According to the definition (Vysotsky, 1923), reconstruction of the rooted vegetation on land plots where it was destroyed by artificial means (timber harvesting, development of land plots for agricultural purposes) or as a result of any natural disasters (attacks of pests, floods, fires) is a demutation process. Intensive overgrowing of hayland, pastures and abandoned land plots represents an initial stage in reconstruction of the natural vegetation cover.

Based on the earlier conducted research (Kechaikina, 2011; Myasnikova et al., 2013; Gorbov et al., 2016; Shchur et al., 2016) it is found out that, in general, uncultivated soils start to resemble similar virgin soils in a number of attributes. However, a damage caused to them is not eliminated within a long period of time. Owing to works of a number of scientists (Myasnikova et al., 2013; Shchur et al., 2016) it is known that reconstructive processes in postagrogenic soils do not stop 80 years after the arable regime. Succession changes of the initial years of the arable regime foster regeneration in arable soils: under the grassland vegetation, former arable horizons undergo transformation according to the sod type (Kechaikina, 2011), the soil density drops, penetration resistance and moisture grow, water permeability increases (Adhikari, 1989; Rao et al., 2014; Xiao et al., 2018), microbial population thrives. Soil development is accompanied by a growing content of organic matters (Shchur et al., 2016), which results in an increased amount of enzymes produced by microorganisms as well as improved immobilization capacity of soils (Khaziev & Gulko, 1991; Bandick & Dick, 1999; Acosta-Martinez & Tabatabai, 2000; Raiesi & Salek-Gilani, 2018).

The research was conducted with a purpose of studying changes in physical properties of arable chernozems under the grassland vegetation in the south of Russia during the first years of the arable regime.

MATERIALS AND METHODS

To observe changes in physical properties of postagrogenic chernozem, a test field was chosen that was located in the botanical garden of the South Federal University. The botanical garden is situated in the center of Rostov-on-Don city in the southwest of the Russian Federation. The research territory has a moderate continental climate. The average annual precipitation is 424 mm. Precipitation occurs mainly in the areas affected by cyclones along the weather fronts. The average air temperature is -7 °C in January and + 23 °C in July.

Profile of Chernozems is made of two main horizons, which are the humus and illuvial-carbonate ones.Typical features for the Chernozems are accumulation of great amount of haumate-calcic humus (up to 15%), good structurization, favorable water-physical properties, saturation with bases, neutral and weak alkaline reaction, stability of mineral soil mass (Glazovsky & Zaitseva, 2009). In 2016 the test field represented an arable plot being under black fallow condition. In spring, prior to the first plowing, the plot was divided into two parts. The first part ceased to be tilled and was converted into an abandoned plot with a purpose of restoring its biological properties. The second part was continued to be tilled, with data from that part further used as a negative control. Two abandoned plots located in the botanical garden (aged 27 and 72 years) and a virgin steppe plot in the Persianovskaya steppe natural sanctuary were used as positive control samples.

Observations were performed within 3 years from 2016 to 2018. A geobotanical description of the plot vegetation was made on 18.06.2016, 25.06.2017, 13.06.2018. Physical properties of the test plots were studied in three periods every year: in May (the active vegetation maximum), end of July (a drought season) and in September (a chilly weather period). Moisture of the test plots was measured in the field with DATAPROBE moisture meter, with 10 replications to a depth of 10 cm. Temperature of the soil surface was measured with HANNA CHECTEMP electronic thermometer (soil surface, 0 cm). Density of the test plots surface was defined by a gravimetric method in triplicate (top soil, 0–10 cm). Soil penetration resistance was defined in the field with Eijkelkamp penetrometer to a depth of 50 cm with 5 cm interval, with 10 replications.

Statistical data processing was performed with the correlation analysis and analysis of variance applied. Statistically significant difference at $p < 0.05$ was taken into account during discussion of the results.

RESULTS AND DISCUSSION

Flora of the abandoned plot in the first year of the research was represented by 9 species. The sward of the postagrogenic plot in the first year of the arable regime was dominated by weedy tallgrass plants: *Ambrosia artemisiifolia; Artemisia vulgari; Cyclachaena xanthiifolia; Chenopodium album*. *Asteraceae* family was dominating in this period (96% of the total species), the remaining families accounted for 4% of the total species. The total biomass on the test plot amounted to 0.76 $g \times cm^{-3}$, the biomass value in the second year dropped by 10% ($p < 0.05$). The flora composition of the plot at the time of research in 2016–2018 and the projective cover of every species are represented in Figs 1, 2. *Asteraceae* family became dominating in the second year of the arable regime (45% of the total species). *Poaceae* grass family was detected as well (10% of the total species). The remaining families were represented by single species (45% of the total species). Flora of the test plot in the second year of the arable regime comprises 21 species.

Flora of the test plot in the third year of the postagrogenic regime comprised 38 species; if compared to the first year, the phytomass value decreased by 26% ($p < 0.05$), with that fact demonstrating a drop in the weedy tallgrass plants domination in the sward. An inverse correlation was detected between the phytomass of the test plot, on one hand, and duration of the arable regime ($r = -0.97$) and quantity of certain plants species ($r = -0.98$), on the other hand. In percentage terms, the plant community was dominated by such families as: *Asteraceae* (33%), *Poaceae* (14%), *Brassicaceae* (6%), *Apiaceae* (6%), *Polygonaceae* (6%), with the remaining families accounting for 36%. *Brassicaceae* (53.5%), *Asteraceae* (23%), *Poaceae* (10%), and *Aceraceae* (2.5%) families prevail in the community in terms of their percentage in the projective cover, whereas other families account for 11.5% of the community. The same plant families prevail in the community in terms of their percentage in the projective cover, but their content differs as follows: primary rooted rosette perennial grass plants constitute 55%, annuals make 17%, creeping stem and deep-rooted perennial grass plants account for 9%, large deciduous trees and scrubs amount to 5%, as well as annual and biennial grass plants account for 4% and perennial grass plants make 4%, whereas other life forms constitute 7%.

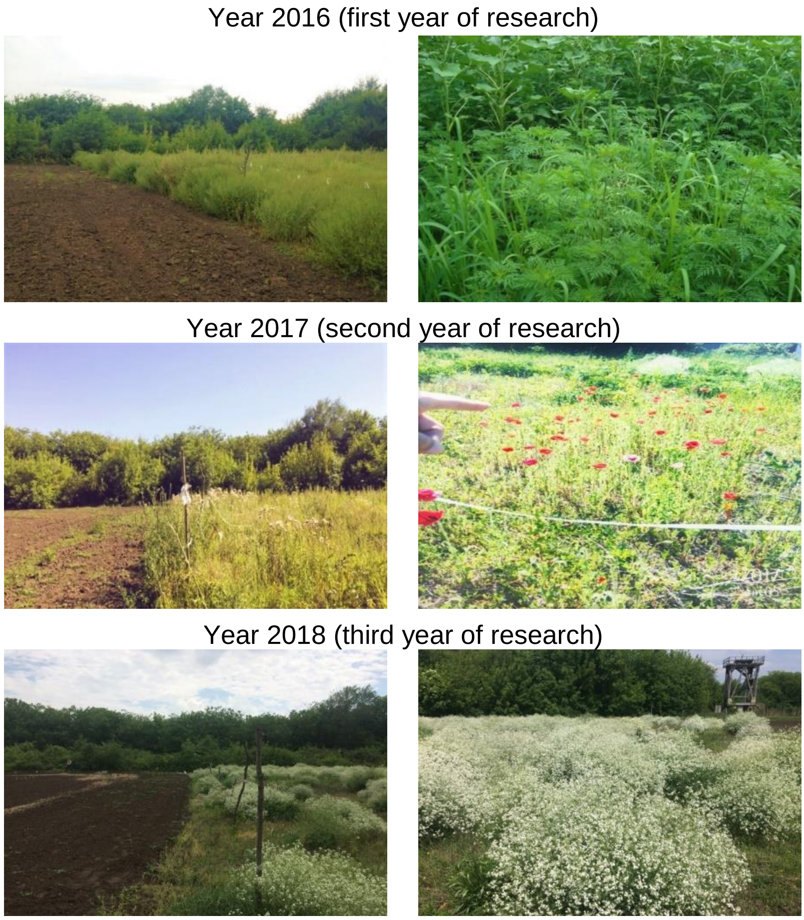


Figure 1. Changes in the floristic composition of the postagrogenic site during four years of research.

Steppe vegetation signs are less evident due to abundance of synanthropic plant seeds. A significant part played by *Ambrosia artemisiifolia* denotes the initial stage of the successive demutation. A dynamics is revealed towards domination of creeping stem and deep-rooted perennials along with a growing projective cover made of hardy-shrub plants. Due to the plot adjoining the forest plantations, there is an ecotonic effect traced in the form of trees and shrubs undergrowth. Subject to absence of the human interference, this factor will determine the future of the plot. After a while, a stable hardy-shrub phytocenosis will be formed in such conditions on the plot.

With appearance of vegetation on the postagrogenic plot in the first year, the soil moisture increased by 8% ($p < 0.05$), if compared to the moisture of the arable land.

Difference in moisture of the tested soil on the arable plot vs. postagrogenic plot grew progressively with every year of research; in 2018 this difference amounted to 17%

($p < 0.05$) (Table 1). Along with moisture growth and vegetation development, temperature on the surface of the test plot fell by 5% ($p < 0.05$), unlike the arable plot temperature.

A tendency of the moisture growth and the fall in temperature on the soil surface of the newly abandoned plot, if compared to the same parameters of the arable plot, was traced within the entire research period and was characterized by an inverse correlation (r = -0.77). It is connected to the vegetation development on the abandoned plot and a further litter formation, which results in shading of the soil, keeping it away from the sunlight. The temperature of the study area in the following years: 1.1–3.1–1.9 °C, no clear trend; however, always the temperature on young fallow is lower than on arable soil. In addition to that, cessation of a regular shuffling of soil during its tillage that entails drying of the surface layer, results in restoration of its structure as well as improvement of its air and water regime. The soil density is influenced by a proportion of organic and mineral matters (Myasnikova et al., 2013), structural aggregates, an amount of roots and a soil tillage degree. Within three years of the abandoned condition the density of the test plot dropped by 9% ($p < 0.05$), if compared to the negative control plot. Owing to the plant roots development, the soil density on the abandoned plot decreased by 11% ($p < 0.05$) during the first year of research. In the third year of research a difference in density between the postagrogenic plot and the steppe plot amounted to 22% ($p < 0.05$). Looking on the soil density (Table 1), there is the following difference between arable and young fallow: 0.15–0.13–0.10 kg×m^{-2} in subsequent years. It means the difference was the largest in 1st year and then continuously decreased. This is due to changing climatic conditions during the study period.

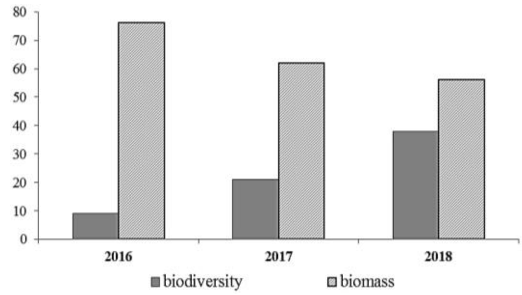


Figure 2. Dynamics of changes of the number of plant species and biomass (100 kg×ha^{-1}) on the fallow after the stop of tillage, 2016–2018.

Table 1. Parameters of the test areas

Research site	Soil density (g×cm^{-3})	Soil surface temperature (°C)	Moisture (%)
2016 year			
Arable	1.32	22.2	18.7
Young fallow	1.17	21.1	20.2
Steppe	0.95	19.7	32.1
2017 year			
Arable	1.29	23.9	14.1
Young fallow	1.16	20.8	16.1
Steppe	0.86	20.3	26.2
2018 year			
Arable	1.30	33.7	11.7
Young fallow	1.20	31.8	13.7
Steppe	0.94	29.7	15.1

An outgrowth of pioneer plants in the first year of the arable regime resulted in changes in a hydrothermal and physical condition of the tested soil. The soil penetration resistance reflects impedance encountered by the root system of plants during their growth. Minimum values for all years of research were detected on the arable plot. During the first year of research there was no significant difference in 0–10 cm plowed horizon of the arable plot vs. the newly abandoned plot (Fig. 3). Along with the development of the plants root system, the soil structure changes as well. A significant difference in penetration resistance of the upper soil layer of the arable plot vs. the abandoned plot was traced in the second year of research, at the depth of 0–20 cm, namely: 0.21 MPa on the arable land and 0.79 MPa on the newly abandoned plot.

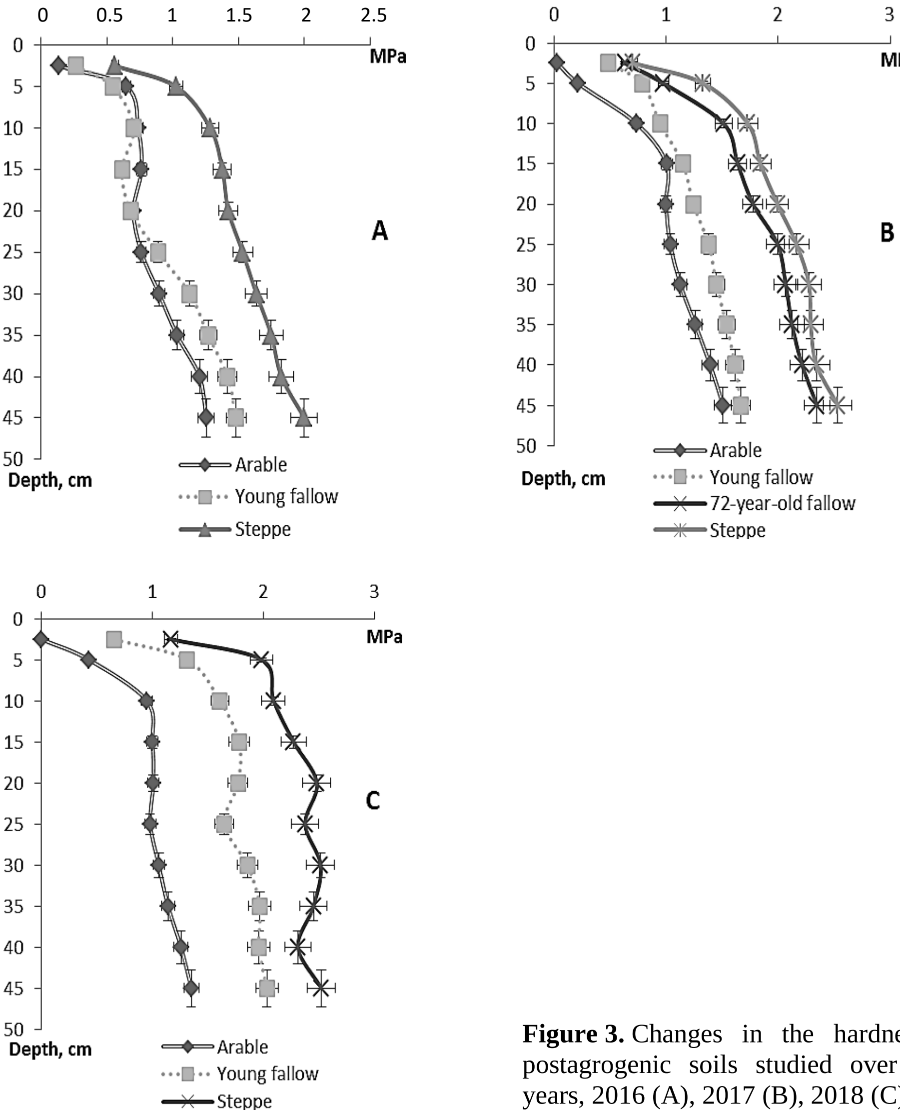


Figure 3. Changes in the hardness of postagrogenic soils studied over three years, 2016 (A), 2017 (B), 2018 (C).

A difference between those two plots has been increasing with the lapse of time. Within the framework of our research a positive correlation was detected between the chernozem density and its penetration resistance (r = 0.70) and temperature (r = 0.73), whereas an inverse correlation was detected between the chernozem density and its moisture content (r = -1.0). The same dependency patterns were revealed by other authors, who studied chernozems of various uses (Myasnikova et al., 2013).

CONCLUSIONS

A physical condition of postagrogenic chernozems begins to change from the first years of the arable regime. The indexes of physical properties of young fallow (during the first 3 years after abandonment) are better than in arable soil and worse than in steppe soil. In the upper horizon, being the most degraded, the soil temperature and density drop, whereas the soil moisture and penetration resistance grow.

ACKNOWLEDGEMENTS. The study was performed with the support of the Ministry of education and science of the Russian Federation (5.5735.2017/8.9) and the President of the Russian Federation (NSH-3464.2018.11).

REFERENCES

Acosta-Martinez, V. & Tabatabai, M.A. 2000. Enzyme activities in a limed agricultural soil. *Biol. Fertil. Soils.* **31**(1), 85–91.

Adhikari, K.R. 1989. *Effect of compaction on physical properties of soil and yield of wheat*. M.Sc. (Hons.) Thesis, Department of Soil Science, Univ. of Agri., Faisalabad.

Bandick, A.K. & Dick, R.P. 1999. Field management effects on soil enzyme activities. *Soil Biology and Biochemistry* **31**, 1471–1479.

Gorbov, S.N., Bezuglova, O.S., Abrosimov, K.N., Skvortsova, E.B., Tagiverdiev, S.S. & Morozov, I.V. 2016. Physical Properties of Soils in Rostov Agglomeration. *Eurasian Soil Science* **49**(8), 898–907.

Glazovsky, N. & Zaitseva, N. 2009. Environmental structure and function: Earth system. EOLSS publishers/UNESCO. 435.

Kalinina, O., Giani, L., Goryachkin, S.V. & Lyuri, D.I. 2015. Postagrogenic development of vegetation, soils, and carbon stocks under self-restoration in different climatic zones of European Russia. *Catena* **129**, 18–29. doi/10.1016/j.catena.2015.02.016

Kazantseva, T.I., Bobrovskaya, N.I., Pashchenko, A.I. & Tishchenko, V.V. 2008. Dynamics of vegetation of 100-year steppe Deposit (Stone steppe, Voronezh region). *Botanical journal* **93**(4) 620–633.

Kechaikina, I.O., Ryumin A.G. & Chukov, S.N. 2011. Postagrogenic transformation of organic matter in soddy-podzolic soils. *Eurasian Soil Science* **44**(10) 1077–1089.

Kudeyarov, V.N. 2019. Soil-biochemical aspects of the state of agriculture in the Russian Federation. *Eurasian Soil Science* **1**, 109–121.

Khaziev, F.Kh & Gulko, A.Ye. 1991. Enzymatic activity of soils under agrocoenoses: status and problems. *Eurasian Soil Science* **8**, 88–103.

Myasnikova, M.A., Ermolaeva, O.Yu., Kazeev, K.Sh. & Kolesnikov, S.I. 2013. Biological features of different age postagrogenic chernozems rostov region. *Modern problems of science and education* **6**, 722–729 (in Russian).

Raiesi, F. & Salek-Gilani, S. 2018. The potential activity of soil extracellular enzymes as an indicator for ecological restoration of rangeland soils after agricultural arable. *Applied Soil Ecology* **126**, 140–147.

Rao, M.A., Scelza R., Acevedo F., Diez M.C. & Gianfreda, L. 2014. Enzymes as useful tools for environmental purposes. *Chemosphere* **107**, 145–162.

Shchur, A.V., Vinogradov, D.V. & Valckho, V.P. 2016. Effect of different levels agroecological loads on biochemical characteristics of soil. *South of Russia: ecology, development* **11**(4), 139–148.

Vysotsky, G.N. 1923. On the prospects of our steppe field and cattle breeding. *Proceedings of the Bureau of applied botany and breeding* **13**, pp. 3–20.

Xiao, W., Chen, X., Jing, X. & Zhu, B. 2018. A meta-analysis of soil extracellular enzyme activities in response to global change. *Soil Biology and Biochemistry* **123**, 21–32.

Agronomy Research **17**(6), 2445–2454, 2019
https://doi.org/10.15159/AR.19.213

Powder particle flow acceleration methods for simulation of interaction with materials used in spacecrafts

Y. Usherenko[1,*], V. Mironovs[1], V. Lapkovskis[1], S. Usherenko[2] and V. Gluschenkov[3]

[1]Riga Technical University, Civil Engineering Department, Scientific Laboratory of Powder Materials, Kipsalas str 6A-110, LV-1048 Riga, Latvia
[2]Belarusian National Technical University, Mechanical Engineering Faculty, Department of Powder Metallurgy, Welding and Material Production Technology, Nezavisimosty Ave., 65, BL220013 Minsk, Belarus
[3]Samara University, Moskovskoye shosse, 34, NIL-41, RU443086 Samara, Russia
[*]Correspondence: osher_yu@mail.ru

Abstract. In recent decades, the role of satellites for monitoring the condition of agricultural land and forests, as well as in the study of natural resources, has especially increased. The amount of debris in near-Earth space is constantly increasing, which creates a real danger to the operation of satellites and other flying objects. The failures of satellites and spacecrafts increase the cost of their production and inhibit the development of the industry, lead to pollution of near-earth space by space debris. The U.S.-based Space Surveillance Network is currently tracking about 40,000 space objects- the vast majority of which are defunct satellites and fragments from collisions. It was estimated that there are more than 8,378 tons of junk around the Earth at speeds of up to 70 km h^{-1}, threatening functioning spacecrafts. Development of a new method for ground-based testing of protective materials, microchips and control systems will enable to avoid further pollution of near-Earth space.
This paper discusses methods for accelerating fine particles using explosive devices and an electromagnetic field and the possibility of using them to develop and research protective materials.

Key words: dynamic impact, shielding, protection, powder metallurgy, spacecraft, space dust, super-deep penetration.

INTRODUCTION

The interaction of hypervelocity particles with the surface of spacecrafts was in the focus of attention since the beginning of regular launches starting from first spacecrafts Vega-1 and Vega-2 and continuing by ESA spacecrafts Giotto and SPADUS. Zodiacal cloud observations and measurements made with a spaceborne dust detector indicate a daily mass input of interplanetary dust particles ranging from 100 to 300 tones (Bernhard et al., 1995; John M.C. Plane 2012; Belous et al., 2019). Thereto more than 5,000 launches since the start of the space age, each carrying satellites have resulted in space becoming increasingly congested and contested.

For a long time, all attention in the development of protective shells is paid to space debris ranging in size between millimetres to meters. However, in the last decade, researches have shown that the influence of space dust (micron-sized) dust has been seriously underestimated. Previous analysis of the state of solar panels the Mir space station, the Hubble Space Telescope and other spacecraft, showed that the layered structures of solar cells are very sensitive to streams of cosmic dust (Rival et al., 1997; Lorenz, 1998). The impact of a superfast microparticle with a velocity $v > 5$ km s^{-1} on a solid target causes mechanical and plasma processes. A crater, an ejection of plasma and steam are forming on the surface of the solar cells, and a shock wave propagates in the battery's layered structure.

The further experiments suggested that when microparticles collide with a spacecraft shield, it may cause not only a mechanical damage, but also causes an occurrence of plasma, which in turn leads to the appearance of electromagnetic radiation capable of disabling nearby electrical equipment (Maki et al., 2004; Fletcher & Close, 2017). Other research has shown that the flow of high-speed dust particles penetrates into metal barriers to depths up to centimetres (Aleksentseva & Krivchenko, 1998; Krestelev, 2014; Qi & Chen, 2014). Therefore, the existing protective shields of spacecraft do not provide reliable protection. Development of additional protection is required. An additional challenge is the carrying out full-scale tests to measure protective properties of various materials. Providing such experiments on spacecraft is costly and complex, so the computer simulation is often used, which is not always able to ensure full compliance.

The effect of super-deep penetration (SDP), demonstrating the possibility of penetration of high-speed flows of powder particles into barriers to depths of tens of centimeters, is capable of creating a ground-based method for testing the protective properties of materials. For this reason, it is necessary to analyze the capabilities of various particle acceleration methods.

SUPER-DEEP PENETRATION METHOD

Super-deep penetration (SDP) is a complex physical phenomenon, when in a split second bunch of powder particles with a fraction less than 200 microns, accelerated to speeds of 700–3,000 m s^{-1}, penetrates into the solid metal body at depth in tens, hundreds mm. At the same time the high and ultra-high pressure (0.2–20 GPa), intensive deformation, local heating, friction are occurred (Kheifets et al., 2004; Owsik et al., 2008).

Penetration depths depend on the material of obstacle as well as on the parameters of a dust cluster. Pressure level in zones of particles motion ensures dynamic phase transition. Material in a solid phase undergoes a transition into a quasi-liquid (dense plasma) state. Due to pulsation of channel elements (in a direction perpendicular to particle motion), plasma jets of opposite directions are formed in channel areas. Jets move inside a shell and ejected from it (Fig. 1). Intense interaction of dust clusters with a protective shell material leads in a closed volume to strong electric effects, which can initiate magnetic field oscillations. During the SDP process in the range of very short intervals flow of powder particles (strikers) move in a volume of the metal body. Behind the strikers in a dense ‘quasi-liquid’ plasma channel cavities are formed, which can slam under the effect of background pressures. In this case matrix crystal lattice is destroyed with high speed, and the matrix material changes from a solid state to a dense plasma, i.e. a dynamic phase transition is realize (Usherenko, Usherenko, and Yazdani 2017). A principal reason of making difficult detection of SDP on space stations is the absence of reach-through holes and, accordingly, absence of depressurization of the module with the equipment.

Under the influence of all these factors, the structure of the matrix material in the areas of ultra-high pressure is ground up to complete amorphisation. These areas are intertwined with the areas of the matrix material, creating in a polycrystalline matrix reinforcing carcass and accordingly an anisotropic composite material (Usherenko et al., 2017). This physical phenomenon occurs only in a closed system.

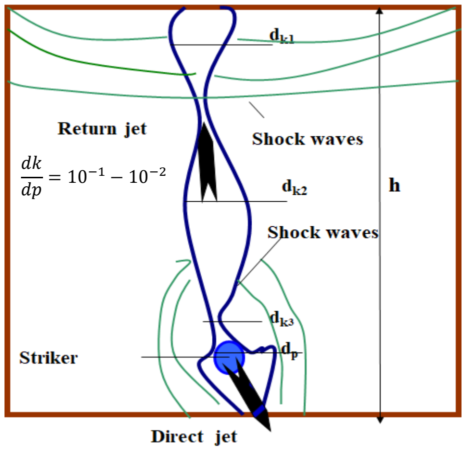


Figure 1. Scheme of channel element (dp – diameter of a striker (particle); dk – diameter of a channel, and h – channel depth).

To develop of new solutions for spacecrafts protection of high-speed dust particles it is necessary to investigate different dynamic load methods, which enable realization of the SDP process in the earth-based conditions.

POWDER PARTICLES ACCELERATION METHODS

Various sources of acceleration of small-sized particles in vacuum and various environments are used for initiation of high-speed movement. These methods can be subdivided into several groups: explosive, electromagnets, etc. Small-sized particles accelerators are subjected to a certain set of requirements, the main ones being simplicity, economy, reliability and safety. For an experimental study of the processes implemented in high-speed collisions, it is necessary to choose an appropriate accelerator providing acceleration of microparticles of a certain mass to given speeds.

A lot of methods are used for throwing bodies of small mass from less than a gram to tens and hundreds of grams. Throwing via resilient elements - strings, rods, curved plates does not provide high throwing speed, due to the complexity of the mechanical construction, and basically does not exceed the speed of sound.

Throwing with high pressure liquid media. The most commonly used is throwing compact bodies or a dispersed medium with water jets. Acceleration of bodies by this method is accompanied by moistening in the throwing medium, additional electrification, and also additional voltage from hydrodynamic flow around the medium (Aleksentseva, 2015; Ben-Dor et al., 2018).

The method of acceleration of micron-sized metallic charged particles using electrostatic accelerators is used for simulating the interaction of solar cells and other elements of a spacecraft with ultrahigh-speed micron particles (micrometeors) (Burchell et al., 1999; Hasegawa et al., 2001). This method provides the acceleration of metal particles with a mass 10^{-10}–10^{-16} g to speeds of tens of km s^{-1}. The disadvantage of this method is that the acceleration of the dielectric microparticles to such speeds is difficult, because charging in contact with a charged needle at the source of micron particles of the accelerator will be in several orders of magnitude lower than charging of metal particle of the same size and mass. Therefore, the speed of the accelerated dielectric particle will be significantly lower than of a metal particle.

In electromagnetic railguns, particles are accelerated by the interaction of induced eddy currents with a moving magnetic flux. With the additional use of explosive compression of the magnetic layer, it is possible to bring the speed of particles with a mass of 0.01 g to 10 km s^{-1} or more (Rashleigh & Marshall, 1978; Semkin et al., 2015). Disadvantages of such railguns are the occurrence of the arc discharge at the contacts, the frequent destruction of the accelerating coils and particles during the acceleration process.

To bring the useful payload into space, electrothermochemical and combined electrodynamic accelerators have been developed. Electrothermochemical accelerators provide speeds up to 2.5 km s^{-1}, electrodynamic accelerators in experiments provide speed about 7 km s^{-1}, but additionally consume energy of hundreds of MJ becoming very expensive equipment for industrial applications (Aleksentseva, 2015).

The magnetic-pulse method is widely used for throwing bodies with a mass of 0.1–500 g at speeds of 30–1,000 m s^{-1} (Mironov & Viba, 2007; Mironovs et al., 2013). The technology of magnetic-pulse processing of materials (MPPM) uses the principle of converting electrical energy stored in a storage device into a powerful pulsed magnetic field that affects the material being processed in a strictly metered form (Gafri et al., 2006; Mironov et al., 2019). For MPPM technological processes, it is necessary to create strong magnetic fields with a strength of 10^5–10^7 A m^{-1}. Such fields are formed in the working tool - inductor when the capacitive energy storage device is discharged onto it (Gluschenkov, 2013). A unit for magnetic-pulse treatment (MPU) generates pulse currents, the magnitude and duration of which can vary widely. The working range of the amplitude of current pulses is 10^4–10^6 A, the duration is 10–1,000 microseconds.

Magnetic pulse installation is a generator of single-multiple current pulses, which contains the energy storage. The stored energy in MPU is released in the inductor-workpiece system in the form of a magnetic field and heat, which are used to perform treatment. High rates of energy release and energy density are realized in pulse modes, which are not achievable in stationary modes, for example, in generators operating at a frequency of 50–1,000 Hz. In MPU during 1–10 seconds energy is accumulated at a relatively low power consumption from the power supply. Depending on the stored energy and efficiency, the average electricity consumption of the MPU from the power network is 0.5–3 kW h^{-1}.

Gas throwing is most widely used for all research purposes. The simplest method is to throw a piston pusher in a tube, which does not provide significant throwing speeds. Pressure P 'can vary in the range of 1–10 MPa. Throwing velocity is in a range of 0.1– 100 m s^{-1}. Two-stage light-gas guns, forming the gas phase in the first stage, provide throwing with a maximum velocity of up to ~7,000 m s^{-1}. The pressure in the gas front at the maximum is up to 1,000 MPa (Aleksentseva, 2015). The three-stage light-gas guns are capable to accelerate projectiles up to 9.5 km s^{-1} (Piekutowski & Poormon, 2006; Putzar & Schaefer, 2016).

Acceleration and throwing of compact bodies by products of explosive combustion. The speed range of explosive combustion is about 3–4.5 km s^{-1}, up to 9 km s^{-1}. The pressure at the front of P ″ can be 1–10 GPa. Explosive combustion is realized by initiating of the propelling charges of gunpowder, high-energy rocket fuels. Explosive methods are the most effective, providing acceleration of bodies due to the initiation of explosives with a detonation velocity D = 5–9 km s^{-1} and pressure P = 10–100 GPa (Huneault et al., 2011). At Fig. 2 a front pressure during throwing by gas discharge, explosive combustion and the shock wave of an explosive are shown (Aleksentseva, 2015).

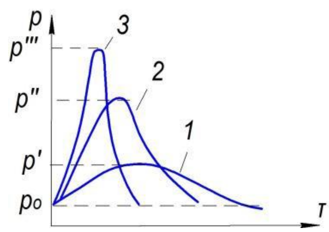


Figure 2. The front pressure (p) at throwing by gas discharge – 1; explosive combustion – 2; the shock wave of an explosive – 3.

Magnetic Pulse Accelerators

At the heart of the principle of MPU (Fig. 3) is used the method of direct conversion of electrical energy stored by the energy storage device into the electromagnetic field arising in the inductor.

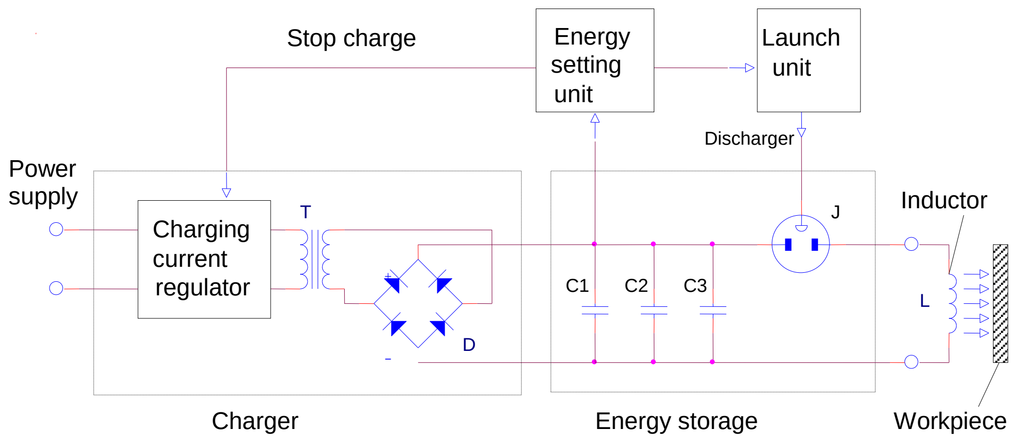


Figure 3. Block diagram of the MPU.

The energy storage device consists of a battery of pulse capacitors C1...C3. Capacitors are charged with direct current from a charger consisting of a high-voltage transformer T1 and rectifier D. The stored energy can be smoothly dosed by adjusting the charge voltage. When the voltage on the capacitors reaches a predetermined level, the energy setting unit stops the charge with the 'Stop' command and issues a discharge command. The start-up unit includes a discharger J, which discharges the capacitors of the energy storage to the inductor L.

In the process of discharge of the energy storage device, an electromagnetic field arises in the working zone of the inductor. The electromagnetic field induces eddy currents in a workpiece. The interaction of the electromagnetic field of the inductor and currents in the workpiece creates a pulse pressure, which leads to the work of deformation and pulse heating of the processed material (Lapkovskis, 2012; Lapkovskis & Mironovs, 2012). The processes of charge and discharge of energy storage in MPU are automated.

The technique features the use of capacitor banks with stored energy from 1 up to 100 kJ. The discharge of capacitors to the coil (working tool) initiates short pulsed electromagnetic field with intensity of 50–200 A m^{-1} and duration of few milliseconds (Fig. 3). The interaction time of flow of particles with a surface of the workpiece is about 2–6 μs. (Fig. 4).

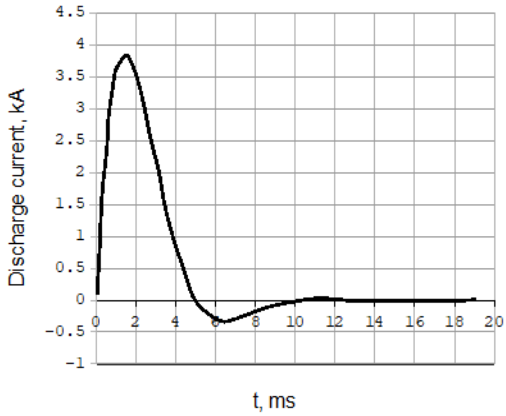


Figure 4. A typical impulse shape generated by impulse current source.

Explosive Accelerators

In accelerators of this type, particles are accelerated by the classical explosive method. There are explosive accelerators of the following types: accelerators with powerful explosives and shaped charges, devices with plasma acceleration and electrostatic accelerators.

Explosive accelerators are characterized by simplicity of design and low cost and are widely used in practice. Acceleration does not depend on the material of the accelerated jet (conductive, non-conductive). When using such accelerators, the flow of microparticles is formed due to the compression of the container with powder particles by the explosive charge (Belous et al., 2017).

The device described in (Usherenko, 2013) can be the basis for creating an accelerator for conducting experiments on modeling and simulating the processes of high-speed collision of cosmic dust with spacecrafts. The equipment is based on the scheme of an explosive accelerator with a cumulative lens. The traditional scheme of the collision, formation and impact of a stream of discrete microparticles is shown in Fig. 5 (Usherenko, 2013). This scheme allows to use relatively small explosive charges

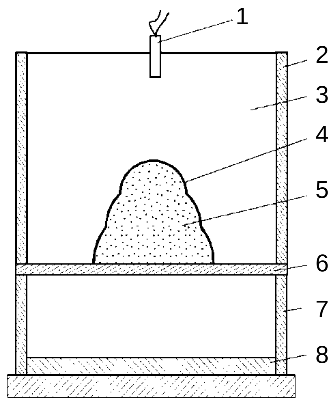


Figure 5. Outdoor explosive accelerator: 1 – initiator; 2 – facing; 3 – charge; 4 – cumulative lens; 5 – powder mixture 6 – plate-cartridge base; 7 – adjusting support; 8 – obstacle.

($\leq$ 0.3 kg), including those from recycled ammunition, with a low detonation velocity ($\leq$ 4,000 m s^{-1}). The average speed of such stream of powder particles is usually ~1,000–3,000 m s^{-1}. The advantages of such scheme are a relatively large mass of the throwing bunch (~0.03–0.2 kg), the ability to control the cross section of the bunch (0.05–0.25 m) and the loading time at colliding with a metal barrier (~10–1,000 μs). At the same time, the high, energy flux has significant gradients of speeds (300–3,000 m s^{-1}) and densities (0.01–0.5$\rho_{theoret}$).

The second variant of the explosive scheme for obtaining the effect of SDP is shown in Fig. 5. In this case, when a gun accelerator is used as the basis, the loading parameters (speed and time) become more defined and constant comparing with a design of an explosive generator (Fig. 6). The speed of throwing the stream according to this scheme is 950–1,200 m s^{-1}. According to the pulse registration data on the oscilloscope during processing, the exposure time of the powder flow with the workpiece was determined (Fig. 7). The interaction time of the particle stream with a total mass of 1 g with the surface of the workpiece was about 10–12 μs. (Aleksentseva, 2015).

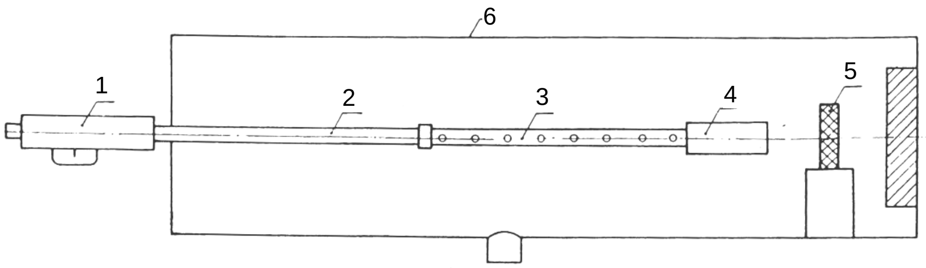


Figure 6. Ballistic accelerator: 1 – charging chamber; 2 – barrel with a diameter of 16 mm; 3 – chamber for depressurization of powder gases; 4 – speed meter; 5 – sample holder; 6 – vacuum chamber.

Explosive methods of throwing in SDP mode in order to ensure throwing velocities in 1.0–3.5 km s^{-1} are the most acceptable for industrial technologies. In addition, high pressures provide an intense impact of the shock wave on the workpiece material, which is a necessary part of the complex super-deep penetration method and to a small extent can be implemented by other methods of throwing. Depending on the goals and objectives, it is possible to apply the scattering of impactors with the necessary spreading radius, formation of a micro-body jet (based on the cumulative effect or a stream of various lengths for processing materials in SDP mode (Aleksentseva, 2015).

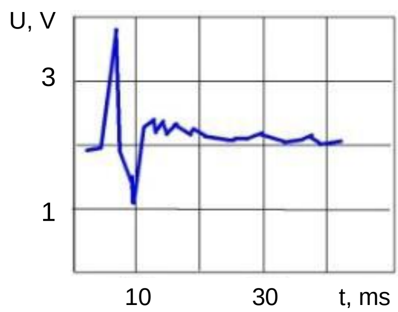


Figure 7. The type of pulse from the pressure sensor when exposed to flow on the workpiece.

The main parameters of explosive and magnetic pulse accelerators of powder particles are given in Table 1. The main advantages of the explosive acceleration method are the high acceleration velocities of the powder flows and amount of energy applied to the accelerated flow. The disadvantages are a small distance of movement and low number of pulses. The main advantages of the electromagnetic acceleration method are a wider range of particle sizes, longer distance of movement and a high number of pulses. A very important advantage of magnetic pulse accelerators is a higher level of staff safety.

Table 1. Parameters of powder particles accelerators

Type of accelerator	Amount of energy applied to the accelerated material (kJ kg^{-1})	Size of the moved particles (mm)	Distance of movement (m)	Velocity of movement (m s^{-1})	Number of pulses (min)
Explosive	150–3,000	0.001–0.5	0.01–0.2	300–3,000	1
Magnetic pulse	2–20	0.001–10.0	0.01–2.0	10–1,000	1–200

CONCLUSIONS

Failures of satellites and spacecrafts increase costs of their production and inhibits the development of the industry, lead to pollution of near, earth space by space debris. The lack of a test method does not allow to solve the problem.

Thus, the investigation of the interaction of high-speed flows of powder particles with a barrier is topical. Using the method of dynamic material loading with a high-speed flow of powder particles will provide an easy and effective way of testing the protective properties of materials and electronic systems in ground-based conditions.

SDP method ensures the penetration of powder particles in metal obstacles to depths of 60–300 mm. It exceeds the thickness of the protective shields of spacecraft. Ground-based materials testing technology developed on the basis of the SDP effect will allow to perform tests of protective properties of various materials using destructive testing tools.

The most promising methods of acceleration in the SDP mode are the magnetic-pulse and explosive methods. Explosive methods of acceleration in SDP mode in order to ensure throwing velocities in 1–3.5 km s^{-1} are the most acceptable for industrial technologies, and magnetic-pulse methods provide greater safety for the staff in laboratory conditions.

AKNOWLEDGEMENTS. This work has been supported by the European Regional Development Fund within the Activity 1.1.1.2 'Post-doctoral Research Aid' of the Specific Aid Objective 1.1.1 'To increase the research and innovative capacity of scientific institutions of Latvia and the ability to attract external financing, investing in human resources and infrastructure' of the Operational Programme 'Growth and Employment' (No. 1.1.1.2/VIAA/2/18/335).

PostDoc Latvia

NACIONĀLAIS ATTĪSTĪBAS PLĀNS 2020

EIROPAS SAVIENĪBA
Eiropas Reģionālās attīstības fonds

IEGULDĪJUMS TAVĀ NĀKOTNĒ

REFERENCES

Aleksentseva, S.E. 2015. *Shock-wave processes of interaction of high-speed elements with condensed media*, Thesis for the degree of Doctor of Technical Sciences. State Technical University of Samara, Samara, Russia, 173 pp. (in Russian).

Aleksentseva, S.E. & Krivchenko, A.L. 1998. Analysis of the Conditions for Ultradeep Penetration of Powder Particles into a Metallic Matrix. *Technical Physics* **43**(7), 859–860.

Belous, A., Saladukha, V. & Shvedau, S. 2017. *Space Microelectronics Volume 1: Modern Spacecraft Classification, Failure, and Electrical Component Requirements*. Artech House, 399 pp.

Belous, A., Saladukha, V. & Shvedau, S. 2019. *High Velocity Microparticles in Space: Influence Mechanisms and Mitigating Effects of Electromagnetic Irradiation*. Springer, 309 pp.

Bernhard, Ronald P., Eric L. Christiansen, James Hyde & Jeanne L. Crews. 1995. Hypervelocity Impact Damage into Space Shuttle Surfaces. *International Journal of Impact Engineering* **17**(1), 57–68.

Burchell, M.J., M.J. Cole, J.A.M. McDonnell & J.C. Zarnecki. 1999. Hypervelocity Impact Studies Using the 2 MV Van de Graaff Accelerator and Two-Stage Light Gas Gun of the University of Kent at Canterbury. *Measurement Science and Technology* 1**0**(1), 41–50.

Fletcher, A.C. & Close, S. 2017. Particle-in-Cell Simulations of an RF Emission Mechanism Associated with Hypervelocity Impact Plasmas. *Physics of Plasmas* **24**(5), 053102-1-053102-7

Ben-dor, G., Dubinsky, A. & Elperin, T. 2018. *Engineering Models In High-Speed Penetration Mechanics And Their Applications (In 2 Volumes)*. World Scientific, 1116 pp.

Gafri, O., Izhar, A., Livshitz, Y. & Shribman, V. 2006. Magnetic Pulse Acceleration. In: *Proceedings of the 2nd International Conference on High Speed Forming*, ICHSF 2006, Dortmund, pp. 33–40.

Gluschenkov, V.A. 2013. *Inductors for Magnetic-Pulse Material Processing. A Tutorial.* Samara: Educational Literature publishing house. Samara: Educational Literature publishing house, 146 pp. (in Russian).

Hasegawa, S., Hamabe, Y., Fujiwara, A., Yano, H., Sasaki, Sh., Ohashi, H. Kawamura, T., Nogami, K., Kobayashi, K., Iwai, T. & Shibata, H. 2001. Microparticle Acceleration for Hypervelocity Experiments by A 3.75MV van de Graaff Accelerator and a 100KV Electrostatic Accelerator in Japan. *International Journal of Impact Engineering* **26**(1), 299–308.

Huneault, J., Loiseau, J., Higgins, A. & Tanguayf, V. 2011. Development of an Implosion-Driven Hypervelocity Launcher for Orbital Debris and Micrometeoroid Simulation, **3**, 2045–2054.

Kheifets, A.E., V.I. Zel'dovich, V.I., Frolova, N.Yu. & Khomskaya, I.V. 2004. A Shock-Wave Model of the Effect of Superdeep Penetration of Powder Particles into Metallic Materials. *Materials Science- Poland* **22**(2), 117–121.

Krestelev, A.I. 2014. Simulation of the Process of Entrainment of a Powder Particles by Exlosive Shock Waves. *Vestn. Samar. Gos. Tekhn. Univ., Ser. Fiz.-Mat. Nauki* **2**(35), 125–29.

Lapkovskis, V. & Mironovs, V. 2012. Single-Stage Electromagnetic Elevator Modelling in FEMM Software. **In:** *Proceedings of the International Conference of DAAAM Baltic*, Tallinn, pp. 321–325.

Lapkovskis, V. 2012. Conveying of Ferromagnetic Powder Materials by Impulse Electromagnetic Field (PhD Thesis). Riga Technical University, 114 pp.

Lorenz, R.D. 1998. Solar Array Degradation by Dust Impacts during Cometary Encounters. *Journal of Spacecraft and Rockets* **35**(4), 579–582.

Maki, K., Takano, T., Fujiwara, A. & Yamori, A. 2004. Radio-Wave Emission Due to Hypervelocity Impacts in Relation to Optical Observation and Projectile Speed. *Advances in Space Research* **34**(5), 1085–1089.

Mironov, V.A., Glushchenkov, V.A., Usherenko, Yu.S., Belyaeva, I.S., Stankevich, P.I. & Irtisheva, I.I. 2019. Manufacture of Products from Boron-Containing Materials by the Method of Combined Static-Pulse Compaction. *IOP Conference Series: Materials Science and Engineering* **558**, 012027-1-012027-3.

Mironov, V. & Viba, J. 2007. Elevator for Powders. **In**: *Congress Proceedings EuroPM 2007*, Toulouse **2**, pp. 39–44.

Mironovs, V., Lapkovsky, V., Kolbe, M., Zemchenkov V. & Shishkin, A. 2013. Use of Pulsed Electromagnetic Fields for Materials Processing in Powder Metallurgy. **In**: *Proceedings of the Conference Euro PM 2013. Compaction.* Gothenburg, **2**, pp. 25–30.

Owsik, J., Jach, K., Usherenko, S., Usherenko, Y., Figovsky, O. & Sobolev, V. 2008. The Physics of Superdeep Penetration Phenomenon. *Journal of Technical Physics* **49**(1), 3–25.

Piekutowski, A.J. & Poormon, K.L. 2006. Development of a Three-Stage, Light-Gas Gun at the University of Dayton Research Institute. *International Journal of Impact Engineering* **33**(1), 615–624.

Plane, J.M.C. 2012. Cosmic Dust in the Earth's Atmosphere. *Chemical Society Reviews* **41**(19), 6507–6518.

Putzar, R. & Schaefer, F. 2016. Concept for a New Light-Gas Gun Type Hypervelocity Accelerator. *International Journal of Impact Engineering* **88**, 118–124.

Qi, C. & Chen, J. 2014. Physical Mechanism of Super-Deep Penetration of Solid Microparticles into Solid Targets. *Journal of the Mechanical Behavior of Materials* **23**(1–2), 21–26.

Rashleigh, S.C. & Marshall, R.A. 1978. Electromagnetic Acceleration of Macroparticles to High Velocities. *Journal of Applied Physics* **49**(4), 2540–2542.

Rival, M., Mandeville, J.C & Durin, C. 1997. Hypervelocity Impacts on Solar Arrays : Analysis of Secondary Particles Ejection and Implications for Environment. *European Space Agency, (Special Publication) ESA SP* (399), 469–75.

Semkin, N.D., Seukhachev, K.I. & Dorofeev, A.S. 2015. Methods and Means of Accelerating Particles of Natural and Technogenic Origin. *Messenger of The Samara University. Aerospace Engineering, Technology and Mechanical Engineering / Vestnik Samarskogo Universiteta. Ajerokosmicheskaja Tehnika, Tehnologii i Mashinostroenie* **14**(4), 171–191 (in Russian).

Usherenko, Yu. 2013. Modification of Metals and Alloys by High-Speed Stream of Solid Particles, Dissertation for the Degree of Candidate of Technical Sciences, 188 pp. (in Russian).

Usherenko, Yu., Usherenko, S. & Yazdani, J. 2017. High-Energy Method of Transformation of Casting Metals and Alloys to the Composite Materials. *Key Engineering Materials* **721**, 290–294.

Agronomy Research **17**(6), 2455–2459, 2019
https://doi.org/10.15159/AR.19.216

Thermoanalytical investigation of selected fuel during isothermal heating

I. Vitázek[1,*], T. Ondro[2], I. Sunitrová[3], R. Majdan[1] and M. Šotnar[2]

[1]Slovak University of Agriculture in Nitra, Faculty of Engineering, Department of Transport and Handling, Tr. A. Hlinku 2, SK949 76 Nitra, Slovakia
[2]Mendel University in Brno, Faculty of Agronomy, Department of Agricultural, Food and Environmental Engineering, Zemědělská 1, CZ61300 Brno, Czech Republic
[3]Constantine the Philosopher University in Nitra, Faculty of Natural Sciences, Department of Physics, Tr. A. Hlinku 1, SK94974 Nitra, Slovakia
[*]Correspondence: ivan.vitazek@uniag.sk

Abstract. The thermal decomposition of woody biomass was studied using pellets made from residual processing spruce wood (*Picea abies*). The samples were studied using thermogravimetric analysis in the isothermal regime at the temperatures 275 °C, 300 °C, 325 °C, and 350 °C, which corresponds to the main decomposition region. The results show that the main decomposition region can be described as a volatilisation of the main constituents at a temperature higher than 300 °C. Otherwise, the results indicate, that the lignin does not decompose at lower temperatures. Therefore, it can be concluded that the heating rate is one of the most important parameters that affect the thermal decomposition of lignin and could lead to different interpretations if non-isothermal measurements are used.

Key words: woody biomass, thermogravimetric analysis, spruce wood.

INTRODUCTION

Wood represents a complex heterogeneous system of various chemical constituents, such as cellulose, hemicellulose, and lignin, along with a small content of inorganic material (Shen et al., 2009; Poletto et al., 2012). According to Mohan et al. (2006), the content of cellulose is ~40–50 wt.%, hemicelluloses ~25–35 wt.% of dry wood. However, softwood comprises ~28 wt.% of hemicelluloses. The content of the last major component, lignin, is 23 wt.%–33 wt.% of softwood.

During heating, in the temperature range of 240 °C–350 °C, the thermal decomposition of cellulose occurs, whereas the hemicelluloses decompose already in the temperature interval of 200 °C–260 °C. The thermal decomposition of lignin takes place between 280 °C–500 °C. However, there is disagreement in the interpretation of the measurements. Orfao et al. (1999), Safi et al. (2004) and Ondro et al. (2018) all stated that the first step of the main decomposition region can be described as a combination of total hemicellulose and cellulose decomposition with partial lignin decomposition. The second step corresponds to the decomposition of the remaining lignin and the combustion of char residues. On the other hand, Bilbao et al. (1997) stated that the first observed step is attributed to the volatilisation of the main constituents, while the second step is assigned to the combustion of char residue. The same explanation for the measured curves was given by both Liu et al. (2002) and Fang et al. (2006). However, some studies show that under certain conditions there should be an interaction between components (Hosoya et al., 2007a; 2007b; 2007c).

These interpretations of the decomposition process could be caused by various factors, such as heating rate, temperature, particle size, pressure, and chemical composition. For the development of efficient biomass combustion applications, it is therefore important to study the thermal decomposition of various kinds of woody biomass.

The aim of this study is to study the main decomposition region using thermogravimetric (TG) analysis during isothermal heating at different temperatures. Based on these results, interpretation about the decomposition of woody biomass that best explains the measured curves can be decided. This paper is a continuation of studies (Ondro et al., 2018; Vitázek & Tkáč, 2019).

MATERIALS AND METHODS

The experiments were carried out using pellets (see Fig. 1) made from the residual processing spruce wood (*Picea abies*) which originated in the locality of Nitra-Horné Krškany, Slovakia. The procedure for producing the wood pellets can be found, for example, in the paper by Holubcik et al. (2012). Due to the influence of particle size on thermal decomposition, an analytical sieve shaker Retsch AS200 was used for sieve analysis. An amplitude of 2.0 mm g^{-1} during the five-minute sieve shaking was applied on sawdust, which was used as the input material for pelletizing. For this analysis, the sieves with opening sizes of 5, 2.5, 1.25, 1, 0.5, 0.2, and 0.1 mm and a bottom pan were used. The material was then put on the sieve with the largest opening size.

Figure 1. Pellets made from the residual processing spruce wood.

The measurements were carried out by a thermogravimetric analyser Mettler Toledo TGA/SDTA 851^e under a dynamic atmosphere of dry air with a flow rate of 50 mL min^{-1} on samples with a mass of ~12 mg.

The decomposition process was described using the non-isothermal measurement in the temperature range of 25 °C–600 °C, with a heating rate of 50 °C min^{-1}. The heating rate of 50 °C min^{-1} was also used to reach the isothermal temperatures from 275 °C to 350 °C. This heating rate ensures that the heat transfer within the sample leads to delay in decomposition, which is needed to decompose as little material as possible. For each isothermal temperature, three or more measurements were carried out and compared in order to ensure the reproducibility of obtained results. The blank measurements with empty crucible were used to subtract the influence of the apparatus on the measurements.

RESULTS AND DISCUSSION

The results of the sieve analysis (see Table 1) show, that only a small amount of material (less than 10 wt.%) was captured by the sieves with opening sizes of 2.5 mm, 1.25 mm, 1 mm and 0.1 mm. On the other hand, more than 30 wt.% of the material was captured by sieves with opening sizes of 0.5 mm and 0.2 mm. Furthermore, the material contains ~7.6 wt.% of particles, with sizes of < 0.1 mm.

Table 1. Sieve analysis of sawdust

Grain size (mm)	Retained (%)
< 5, 2.5)	0.19
< 2.5, 1.25)	6.20
< 1.25, 1)	7.93
< 1, 0.5)	33.99
< 0.5, 0.2)	34.20
< 0.2, 0.1)	9.85
lower than 0.1	7.64

The TG curve and its derivative (dTG) for a heating rate of 50 °C min^{-1} are shown in Fig. 2. The first process, which occurs in the temperature range of 40 °C–150 °C, corresponds to the release of the moisture and adsorbed water, during which the mass loss is ~8%. The second process (250 °C–400 °C) can be characterised as the main decomposition region and is accompanied by a mass loss of ~60%. The last process occurs in the temperature range of 400 °C–570 °C. Similar curves (see Fig. 3) can be found in the study by Cui et al. (2019), where the authors used the nitrogen and oxygen in ratio 8:2 for studying feedstock, which includes the powder of *Chlorella Vulgaris* and apple tree sawdust.

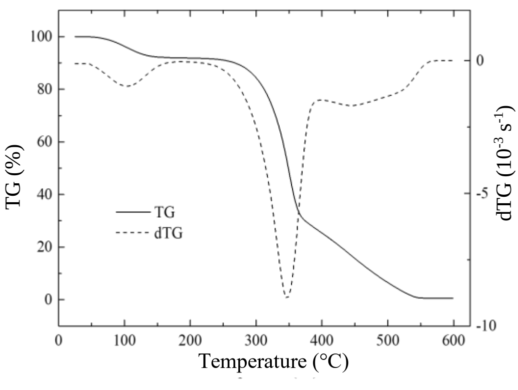


Figure 2. TG and dTG curves for heating rate 50 °C min^{-1}.

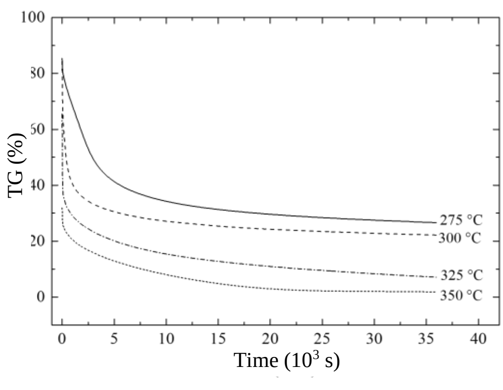


Figure 3. Results of TG analysis under isothermal heating.

The results of the isothermal TG analysis are shown in Fig. 3. The time $t = 0$ corresponds to the point when the isothermal heating began. Temperatures of 275 °C, 300 °C, 325 °C, and 350 °C were chosen due to their correspondence to the main decomposition region. The TG curves show that if higher temperatures are used, then a higher mass loss is observed. In addition, the results show, that the decomposition process is not complete even if the sample is heated at 350 °C for ten hours.

The results show that if the temperature of the isothermal regime is 275 °C and 300 °C, then the total mass loss is ~73% and 78%, respectively. However, if temperatures 325 °C and 350 °C were used, which still correspond to the main decomposition region, the total mass loss is higher (93% and 98%, respectively). As mentioned in Section 1, the softwood contains 23 wt.%–33 wt.% of lignin, which can indicate that the lignin has already decomposed. Therefore, it can be concluded that the main decomposition region can be described as volatilisation of the main constituents if temperatures higher than 300 °C are used. Otherwise, the results indicate, that the lignin does not decompose at lower temperatures. The same interpretation of non-isothermal measurements was also used by Bilbao et al. (1997), Liu et al. (2002) and Fang et al. (2006). On the other hand, non-isothermal measurements could lead to different interpretations. Based on the results in this work, it can be also concluded that the heating rate is, along with the content (Gottipati & Mishra, 2011), one of the most important parameters that affect the thermal decomposition of lignin.

In a recently published article, Vitázek & Tkáč (2019) studied the region between the temperatures of 275 °C and 290 °C. Compared to these results, the region which corresponds to the beginning of the main decomposition region can be described as a first-order reaction model. However, a determined reaction model cannot be used to describe the whole process.

CONCLUSIONS

The thermal decomposition of pellets made from spruce wood (*Picea abies*) was studied using thermogravimetric analysis under the dynamic atmosphere of dry air. As there are different interpretations of the main decomposition region in the literature, the woody biomass was studied under isothermal conditions at temperatures of 275 °C, 300 °C, 325 °C and 350 °C over the course of ten hours. Based on the results it can be concluded, that the possible explanation for the main decomposition region seems to be the volatilisation of the main constituents. Otherwise, the results indicate that lignin does not decompose at temperatures lower than 300 °C. Therefore, it can be concluded that the heating rate is one of the most important parameters that affect the thermal decomposition of lignin and could lead to different interpretations if non-isothermal measurements are used.

ACKNOWLEDGEMENTS. Supported by the Ministry of Education of the Slovak Republic, Project VEGA 1/0464/17 'Monitoring of the impact of ecological fuels obtained from the agricultural production and additives in hydrocarbon fuels to technical and environmental performance of internal combustion engines used in agricultural and transport technique'.

REFERENCES

Bilbao, R., Mastral, J.F., Aldea, M.E. & Ceamanos J. 1997. Kinetic study for the thermal decomposition of cellulose and pine sawdust in an air atmosphere. *Journal of Analytical and Applied Pyrolysis* **39**, 53–64.

Cui, X., Yang, J., Shi, X., Lei, W., Huang, T. & Bai, C. 2019. Experimental Investigation on the Energy Consumption, Physical, and Thermal Properties of a Novel Pellet Fuel Made from Wood Residues with Microalgae as a Binder. *Energies* **12**, 3425.

Fang, M.X., Shen, D.K., Li, Y.X., Yu, C.J., Luo, Z.Y. & Cen, K.F. 2006. Kinetic study on pyrolysis and combustion of wood under different oxygen concentrations by using TG-FTIR analysis. *Journal of Analytical and Applied Pyrolysis* **77**, 22–27.

Gottipati, R. & Mishra, S. 2011. A kinetic study on pyrolysis and combustion characteristics of oil cakes: Effect of cellulose and lignin content. *Journal of Fuel Chemistry and Technology* **39**, 265–270.

Holubcik, M., Nosek, R. & Jandacka, J. 2012. Optimization of the Production Process of Wood Pellets by Adding Additives. *International Journal of Energy Optimization and Engineering* **1**, 20–40.

Hosoya, T., Kawamoto, H. & Saka, S. 2007a. Pyrolysis behaviors of wood and its constituent polymers at gasification temperature. *Journal of Analytical and Applied Pyrolysis* **78**, 328–336.

Hosoya, T., Kawamoto, H. & Saka, S. 2007b. Influence of inorganic matter on wood pyrolysis at gasification temperature. *Journal of Wood Science* **53**, 351–357.

Hosoya, T., Kawamoto, H. & Saka, S. 2007c. Cellulose-hemicellulose and cellulose-lignin interactions in wood pyrolysis at gasification temperature. *Journal of Analytical and Applied Pyrolysis* **80**, 118–125.

Liu, N.A., Fan, W., Dobashi, R. & Huang, L. 2002. Kinetic modeling of thermal decomposition of natural cellulosic materials in air atmosphere. *Journal of Analytical and Applied Pyrolysis* **63**, 303–325.

Mohan, D., Pittman, Ch.U. & Steele, P.H. 2006. Pyrolysis of Wood/Biomass for Bio-oil: A Critical Review. *Energy & Fuels* **20**, 848–889.

Ondro, T., Vitázek, I., Húlan, T., Lawson, M.K. & Csáki, Š. 2018. Non-isothermal kinetic analysis of the thermal decomposition of spruce wood in air atmosphere. *Research in Agricultural Engineering* **64**, 41–46.

Orfão, J.J.M., Antunes, F.J.A. & Figueiredo, J.L. 1999. Pyrolysis kinetics of lignocellulosic materials-three independent reactions model. *Fuel* **78**, 349–358.

Poletto, M., Zattera, A.J. & Santana, R.M.C. 2012. Thermal decomposition of wood: Kinetics and degradation mechanisms. *Bioresource Technology* **126**, 7–12.

Safi, M.J., Mishra, I.M. & Prasad, B. 2004. Global degradation kinetics of pine needles in air. *Thermochimica Acta* **412**, 155–162.

Shen, D.K., Gu, S., Luo, K.H., Bridgwater, A.V. & Fang, M.X. 2009. Kinetic study on thermal decomposition of woods in oxidative environment. *Fuel* **88**, 1024–1030.

Vitázek, I. & Tkáč, Z. 2019. Isothermal kinetic analysis of thermal decomposition of woody biomass: The thermogravimetric study. *AIP Conference Proceedings* **2118.**

Agronomy Research **17**(6), 2460–2473, 2019
https://doi.org/10.15159/AR.19.208

Application of geographically weighted principal components analysis based on soybean yield spatial variation for agro-ecological zoning of the territory

A. Zymaroieva[1,*], O. Zhukov[2], T. Fedonyuk[3] and A. Pinkin[4]

[1]Zhytomyr National Agroecological University, Faculty of Forestry, Department of Forest Resources Utilization, Stary Blvd. 7, UA10008 Zhytomyr, Ukraine
[2]Oles Honchar Dnipro National University, Faculty of Biology and Ecology, Department of Zoology and Ecology, pr. Gagarina, 72, UA49010 Dnipro, Ukraine
[3]Zhytomyr National Agroecological University, Faculty of Forestry, Department of Forest Ecology and Life Safety, Stary Blvd. 7, UA10008 Zhytomyr, Ukraine
[4]Zhytomyr National Agroecological University, Faculty of Engineering and Power Engineering, Department of Electrification, Automation of Production and Engineering Ecology, Stary Blvd. 7, UA10008 Zhytomyr, Ukraine
[*]Correspondence: nastya.zymaroeva@gmail.com

Abstract. In this study, the geographically weighted principal components analysis as an alternative method for agro-ecological characterization of the region was provided. The spatial and temporal distribution pattern of soybean yield was analyzed by using spatial statistics technology, which provided a good reference for agricultural development planning. The soybean yield was selected for the present study because it is a comprehensive indicator reflecting the production potential of the regional agroecosystems. The organized data set, which included the average per year yields of soybean in 10 regions (206 administrative districts) of Ukraine, was used for analysis. The regular temporal trend, specific for each district, was previously extracted from the time series data. The principal components analysis of the detrended data allowed to identify four principal components, which altogether can explain 58% of the soybean yield variation. The geographically weighted principal components analysis allowed to reveal that four spatially determined processes were influencing the yield of soybeans and had the oscillatory dynamics of different periodicity. It was hypothesized that the oscillating phenomena were of ecological nature. Geographically weighted principal component analysis revealed spatial units with similar oscillatory component of soybean yield variation. Our study confirmed the hypothesis that within the studied territory there are zones with the specific patterns of the temporal dynamics of soybean yield, which are uniform within each area but qualitatively different between zones. The territorial clusters within which the temporal dynamics of soybean yield is identical can be considered as agro-ecological zones for soybean cultivation.

Key words: cluster analysis, geographically weighted principal components analysis (GWPCA), soybean, spatial variability, productivity, yield.

INTRODUCTION

Sustainable agricultural development requires a systematic effort towards the planning of land use activities in the most appropriate way. Agro-ecological zoning is one of the cornerstones for agricultural planning because survival and failure of particular land use or farming system in a given region heavily relies on careful assessment of agro-climatic resources (Patel, 2003). A framework of agro-ecological zoning describing concepts, methods and procedures was conceptualized for the first time by FAO (1976). Agro-ecological zoning refers to the division of an area of land into land resource mapping units, having a unique combination of landform, soil and climatic characteristics and/or land cover. (FAO, 1996; Patel, 2003). Therefore, each zone has a similar combination, constraints and potential for the use of land, which serves as the focus of recommendations designed to improve the existing land use, either through increased production or by limiting land degradation (Suriadikusumah & Herdiansyah, 2014). The main objectives of agro-ecological zoning are data inventory of environmental resources, identification of homologous environments, determination of agricultural potential of a region, planning for regional development and identification of research priorities. Conventional methods employed are overlaying of maps and various statistical techniques (Aggarwal, 1991).

Principle components analysis (PCA) is a statistical method widely used in exploratory data analysis (Pearson, 1901). This non-parametric method reduces the dimension of a dataset, which simplifies structures hidden in the dataset (Liu et al., 2012). Principal components analysis has been applied by various researchers' area to explore and characterize the relationships between regionalized variables and related environmental factors, and to quantify the spatial variability pattern of these variables (Kumar et al., 2012). In an ecological setting, common applications of PCA are employed to environmental data sets e.g., the soils biogeochemistry data, species abundance data etc. (Legendre & Gallagher, 2001; Kaspari & Yanoviak, 2009).

Geographically weighted principal components analysis (GWPCA) is a localized version of PCA that is an exploratory tool for investigating spatial heterogeneity in the structure of multivariate data (Harris, 2011). Hence, a GWPCA investigates how outputs from a PCA vary spatially (Comber et al., 2016). Spatial changes in data dimensionality and multivariate structure can be explored via maps of the GWPCA outputs. GWPCA can also be used to detect multivariate spatial anomalies (Harris et al., 2015; Comber et al., 2016). In the published literature, GWPCA has been applied for analyzing multivariate population characteristics (Lloyd, 2010), social structure (Harris et al., 2011), soil characteristics (Kumar et al., 2012) and freshwater chemistry data (Harris et al., 2015, Li et al., 2015). However, GWPCA has not been applied to assess spatial variability of crop yields in agricultural landscapes, moreover, it has never been used for agro-ecological zoning of an area.

In this study, we consider the possibility of applying the geographically weighted principal components analysis as an alternative method for agro-ecological characterization of a region. The soybean yield was selected as the basis for agro-ecological zoning, because it is the comprehensive indicator, reflecting the production potential of agroecosystems (Kukal & Irmak, 2018). Crop yield is influenced by both management and environmental factors, but definite quantitative relationships are not easy to obtain because of complicated interactions between these factors (Ruiz-Vega, 1984). However, if the influence of agro-technological and management factors has the general origin and are described by the regression model, the influence of environmental factors leads to yield fluctuations (residuals) that do not fit into the total trend (Zhukov et al., 2018). These residuals also have a complex nature. There is a random noise associated with objective errors in the source data. However, in the regression residuals, we can expect a component that is associated with a regular variation that has an ecological nature (Kunah et al., 2018). Thus, the study of the residuals of the yield regression model allows us to separate the ecological determinants of soybean yield variation. Besides, through GWPCA it is possible to map areas with similar temporary fluctuations in yield, which may be regarded as agro-ecological zones for soybean cultivation.

The objective of this research was to study the spatial variation of the temporal patterns of the soybean yield. We have discussed two alternative hypotheses. The first one is the spatial variation of the soybean yield is per the uniform trend and there is no interruption of the continuous yield dynamics within the studied territory. The second one is that within studied territory there are zones with specific patterns of the temporal dynamics, which are uniform within each area but qualitatively different between zones.

MATERIALS AND METHODS

Time series of the soybean yields for each administrative district was divided into two components: total trend and trend residual. Total trend was determined by the dependence of the yield on time. As an analytical form of the trend, we selected the fourth-degree polynomial. The residuals of the corresponding regression models that describe the trends consist of the random component (noise) and, probably, the regular one that cannot be explained by the selected trend model. These two components are distinguished by their properties: the random component is an independent one for different points of space, and the regular component must be correlated to all or some points in space (administrative districts). We used the principal components analysis (PCA) for the residuals to isolate the regular component of trend models. The presence of the principal components, whose eigenvalues are more than 1, indicates that there exists a correlation in crop yields variation. Data on the yield of soybean were obtained from the State statistics service of Ukraine (http://www.ukrstat.gov.ua/) and its regional offices. Specifically, the organized data set included the average per year yields of the soybean for 10 regions of Ukraine (Vinnytsia, Volyn, Zhytomyr, Kyiv, L'viv, Rivne, Ternopil, Khmelnytsky, Cherkasy, Chernihiv), which include 206 administrative districts (Fig. 1). Information covers a period from 1991 to 2017.

Principal components analysis (PCA) is widely used for dimensionality reduction of the multivariate data set (Liu et al., 2012). Principal component analysis was performed using library stats (R Core Team, 2017). The suitability of yield data for the principal components analysis was evaluated by the Kaiser-Meyer-Olkin (KMO) test (Kaiser, 1974) with the help of the function KMOS from the library REdaS (Maier, 2015) in the environment for statistical computing R (R Core Team, 2017). Horn's (1965) technique for evaluating the components in a principal components analysis was implemented through *paran* function from the library ‘paran’ (Dinno, 2012). Geographically weighted principal components analysis (GWPCA) may be used to account for spatial heterogeneity in the structure of the multivariate data (Harris et al., 2011). An essential component of the GWPCA modelling is the spatial weighting function that quantifies the spatial relationship or spatial dependency between the observed variables (Fotheringham et al., 2002). A bandwidth for spatial analysis was found optimally using cross-validation with the Gaussian kernel function. Monte Carlo test was performed to examine whether yield data matrix eigenvalues were spatially varying (Iqbal et al., 2005). The GWPCA method is implemented using the GWmodel R package (Gollini et al., 2013). To visualize GWPCA outputs, the spatial distribution of the first four principal components percentage of the total variance was mapped. The locale influence of the variables on principal components 1–4 was visualized by mapping the 'winning variable' with the highest absolute loading. The spatial database was created in ArcGIS 10.0. The spatial autocorrelation, *I*-Moran's statistics (Moran, 1950), was calculated using Geoda095i (Anselin et al., 2005).

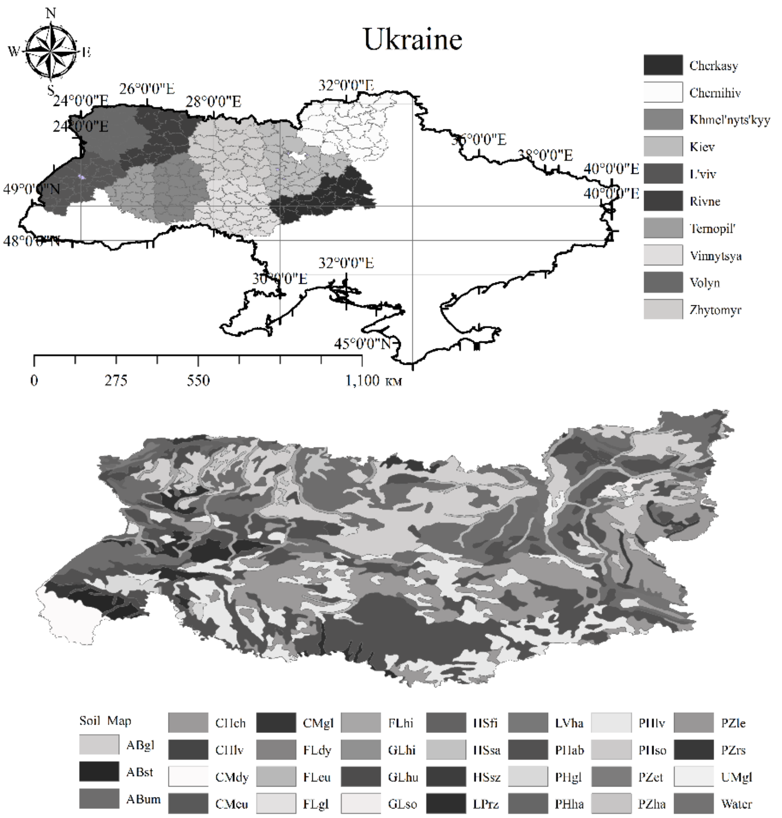


Figure 1. Map of 10 administrative regions in Ukraine, Ecoregions and soil map (Hengl et al., 2017).

Legend: Soil classification according World Reference Base for Soil Resources: ABgl – Albeluvisols Gleyic; ABst – Albeluvisols Stagnic; ABum – Albeluvisols Umbric; CHch – Chernozems Chernic; CHlv – Chernozems Luvic; CMdy – Cambisols Dystric; CMeu – Cambisols Eutric; CMgl – Cambisols Gleyic; FLdy – Fluvisols Dystric; FLeu – Fluvisols Eutric; FLgl – Gleyic Fluvisols; FLhi – Fluvisols Histic; GLhi – Gleysols Histic; GLhu – Gleysols Humic; GLso – Gleysols Sodic; HSfi – Histosols Fibric; HSsa – Histosols Sapric; HSsz – Histosols Salic; LPrz – Leptosols Rendzic; LVha – Haplic Luvisols; PHab – Phaeozems Albic; PHgl – Phaeozems Gleyic; PHha – Phaeozems Haplic; PHlv – Phaeozems Luvic; PHso – Phaeozems Sodic; PZet – Podzols Entic; PZha – Podzols Haplic; PZle – Leptic Podzols; PZrs – Podzols Rustic.

RESULTS AND DISCUSSION

The global principal components analysis

The dissimilar magnitude between regression residuals for administrative areas may lead to biased results from PCA as the variables with the highest sample variances tend to be emphasized in the first few principal components. Hence, all the selected variables need to be standardized by subtracting its mean from that variable and dividing it by its standard deviation. Such data standardization makes each transformed variable have equal importance in the subsequent analysis (Li et al., 2015).

As described before, the total number of 206 units was observed for 27 variables (years). The Kaiser-Meyer-Olkin (KMO) index was run for the overall data set to detect sampling adequacy. As the KMO value is 0.63, according to the Kaiser empirical rule (Kaiser, 1974), the study data should be considered relevant for the principal components analysis.

The PCA of the residuals of the regression model allowed to establish that the number of statistically probable principal components is 4 according to the Horn procedure (Horn, 1965). The four components with eigenvalues larger than 1 explain up to 58% of variation in the regional soybean yield (Table 1).

Table 1. Summary of global PCA

Principal components	Adjusted* eigenvalues	Unadjusted eigenvalues	Estimated bias	Proportion of variance	Standard deviation
1	8.30	9.04	0.73	33.47	3.00
2	2.45	3.08	0.62	11.39	1.75
3	1.33	1.86	0.54	6.90	1.36
4	1.21	1.67	0.46	6.20	1.29

Symbols: *– by Horn's parallel analysis.

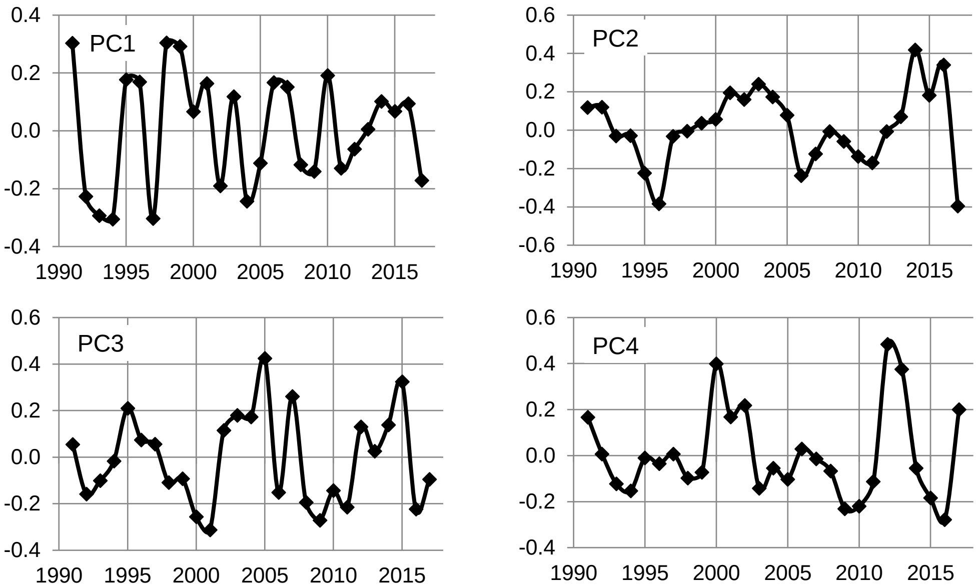


Figure 2. The principal components loadings to the variables.

The variables used in the PCA are the ordinal quantities – the years, so the loadings of the principal components on the variables can be represent ted as dynamic changes in time (Fig. 2). This form of presentation enables us to interpret meaningfully the determined principal components as oscillation processes with different frequencies. Thus, the principal component 1 explains 33.47% of the total variability of the soybean yield. It is characterized by a predominant oscillation process within 5 years. Moreover, this principal component demonstrates a clear trend towards damping of the oscillation process during the study period.

The variation of principal component 1 is spatially determined (*I*-Moran 0.29, $P = 0.001$). The zones with higher values of principal component 1 form clusters in some northern areas of the studied region, as well as in the western ones. The zone with the lower values of principal component 1 forms a cluster in the southeastern direction from the center of the region (Fig. 3).

Principal component 2 explains 11.39% of the total dispersion and as to its fluctuations, most typical is an oscillating process with a lag of ten years. This component demonstrates spatially regular patterns of variation (*I*-Moran 0.48, $P = 0.001$). Clusters with higher values of principal component 2 are located in the south-western and north-eastern regions, and with the lower ones – in the north-west and south-east (Fig. 3).

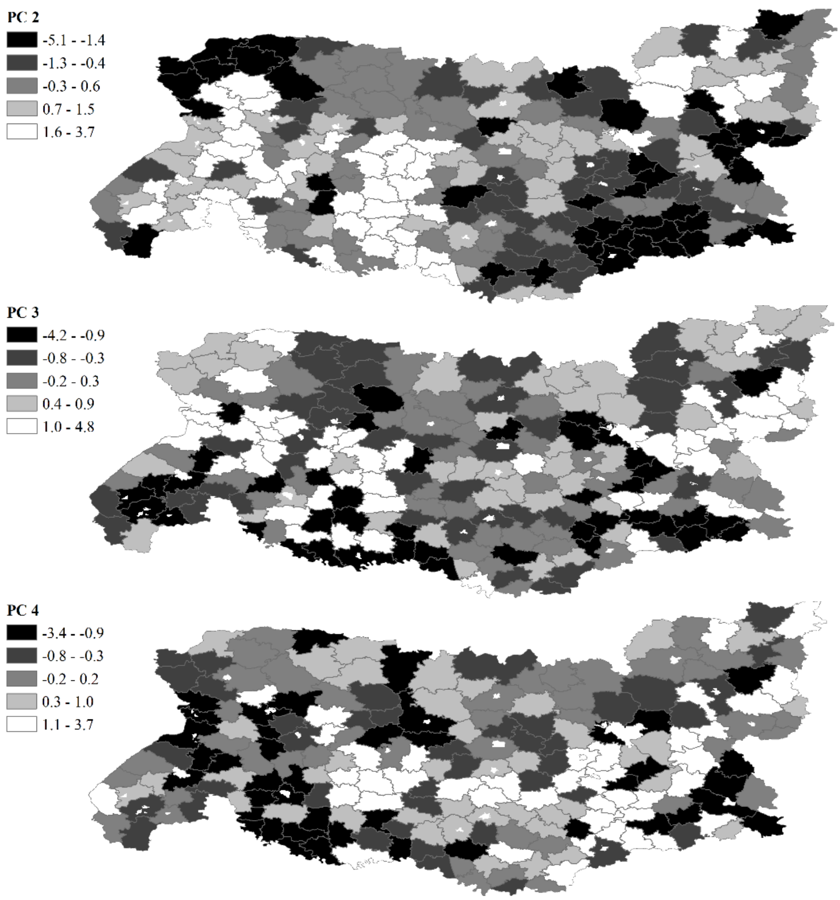


Figure 3. Spatial variation of principal components 1–4.

Principal component 3 explains 6.90% of the soybean yield variability and its characteristic oscillations are repeated every 9-10 years. The high spatial level of the principal component 3 variation is confirmed by *I*-Moran statistics (0.28, $P = 0.001$). Clusters with high values of principal component 3 are typical of the southwest and east, and with low values – of the southeast.

Principal component 4 explains 6.20% of the dispersion of the soybean yield. For its fluctuations in time, the most characteristic period is also a span of 8-9 years (Fig. 2). The spatial patterns of this component are statistically significant (*I*-Moran 0.29, $P = 0.001$). The clusters with the higher values of principal component 4 are characteristic for the center and east of the region, and with lower values – for the west (Fig. 3).

Thus, the global principal components analysis revealed the presence of dynamic processes of soybean yield, which have the oscillatory nature with varying frequencies. We associate oscillatory processes of varying frequency with causes of different nature.

The principal components analysis of the regression model residues of the time trend enables us to prove that within a set of ecological factors four principal components affect the soybean yields to the greatest extent. Specification and detailed research of the origin of these principal components are objective of our subsequent studies. However, at this point we can prove the presence of four spatially determined processes that influence the yield of soybeans and have the oscillatory dynamics of different periodicity.

Geographically weighted principal components analysis

The Monte Carlo test was conducted to examine whether the data matrix eigenvalues are spatially varying ($P = 0.01$). Thus, there is a high degree of spatial non-stationarity present in the data of regional soybean yield.

The previous global PCA results indicate that the first four components can collectively explain 58% of the variance in data structure. Accordingly, it is reasonable to retain the four components for further GWPCA analysis. However, since the paper is limited in scope, only the first two components GWPC 1 and GWPC 2 from GWPCA will be comparatively interpreted in detail.

The results of the procedure GWPCA can be visualized and interpreted by focusing on how the dimensions of the data vary spatially and how the original variables affect the principal components (Li et al., 2015). Percentage of spatial variation of the total variation demonstrates a clearly expressed variability, thus forming spatially homogeneous clusters from north to south of the research region (Fig. 4). Compared with the global analysis of the principal component, GWPCA demonstrates its effectiveness and efficiency in the analysis of spatial patterns of regional placement of soybean yields, using the mapping of spatial variability of the principal components.

It was suggested that the variables with the highest loading values and their impact intensity values can be mapped locally (Lloyd, 2010). Then we can visualize how each of the four variables locally affects the given component, displaying the 'winning variable' with the highest absolute loading. Fig. 5 shows the spatial distribution of variables with the highest absolute loading of GWPC 1–4, respectively.

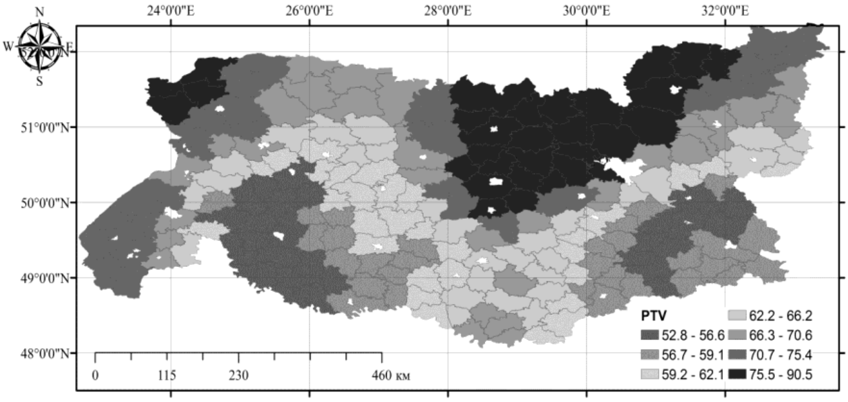


Figure 4. Spatial variability of the percentage of total variance (PTV) of the first four principal components.

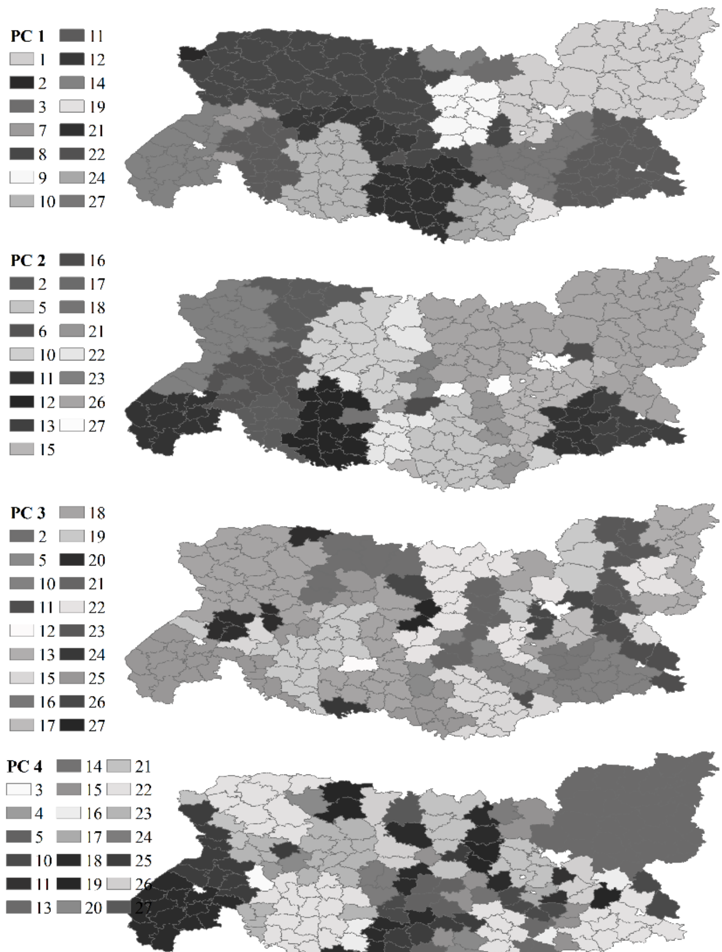


Figure 5. Spatial location of ‘winning’ variables for principal components 1–4.

The traditional representation of 'winning' variables for the principal components cannot fully reveal the nature of the spatially dependent relationship between the indicators estimated by the principal component analysis. The factor of loading predominance is one of the aspects that reflects the crop yields dynamics. Due to the oscillating nature of such dynamics, predominance is the random outlier of the indicator at a certain moment in comparison with the general recurring dynamics. Therefore, for each of the statistically significant principal components, we conducted the classification of administrative districts by cluster analysis based on distance, which is opposite to the Pearson correlation coefficient. This indicator of distance is sensitive to the form of comparable indicators, and not to their absolute values. This approach allows to identify groups of administrative districts with a similar time dynamic of soybean yields in the aspect of the corresponding principal component. It can be assumed that the aggregates of administrative districts with a similar yield's dynamics are also geographically close and form homogeneous ecological regions.

Cluster analysis of the administrative districts by factor loading values of GWPC 1 revealed three homogeneous clusters (Fig. 6). For each cluster, we calculated the average values of the factor loadings, which helped assess the specificity of the respective clusters (Fig. 7). The general trend of principal component 1 is the damping of the amplitude of the oscillations during the research period and the predominance of higher frequency components of oscillatory dynamics corresponding to the heterogeneity of observations over time or heteroscedasticity. So, the Koenker-Bassett test for clusters 1 and 3 indicates the heteroscedasticity of the time dynamics of factor loadings (1.17, $P = 0.28$ and 1.35, $P = 0.24$, respectively). The heteroscedasticity is established for cluster 2 (5.09, $P = 0.024$). Thus, the qualitative feature of the soybean yield dynamics in the corresponding clusters is the difference in levels of damping of the principal component 1 oscillations over time.

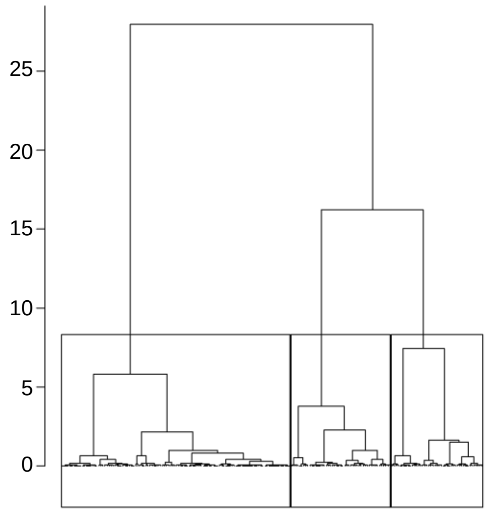


Figure 6. Cluster analysis of administrative districts by factor loadings values GWPC 1.

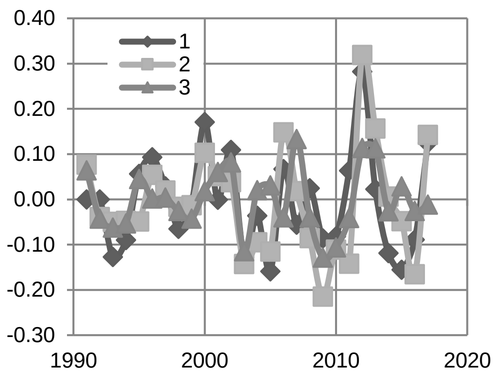


Figure 7. The average values of factor loadings of GWPC 1 for clusters 1–3. Here abscissa is the primary variables (the residuals of the regression models of the trend of yield by years), axis ordinate - factor loadings.

The spatial distribution of administrative districts included in the respective clusters is spatially regular (Fig. 8). Cluster 3 covers the largest part of Ukraine and is located in the north, center and west of the studied territory. Clusters 1 and 2 are located in the south of the research area.

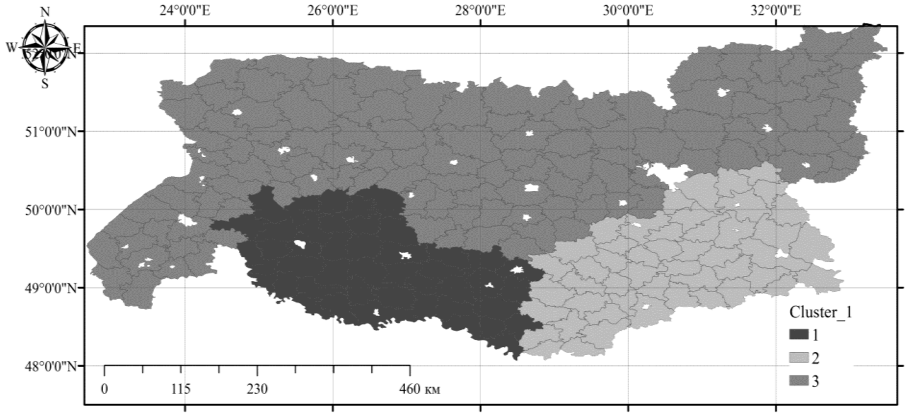


Figure 8. Spatial location of the clusters obtained based on the GWPC 1 loadings.

Cluster analysis of the values of factor loadings GWPC 2 revealed four homogeneous clusters (Fig. 9).

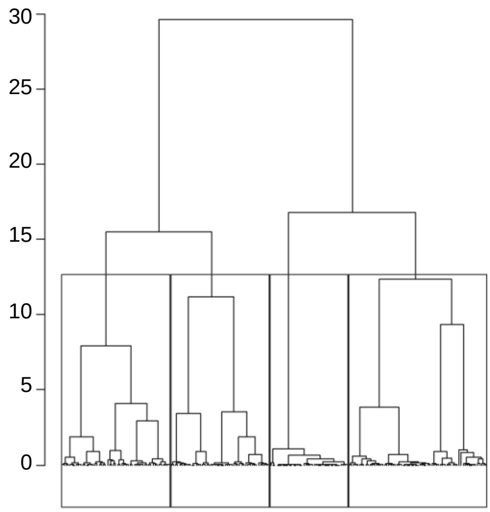


Figure 9. Cluster analysis of administrative districts by factor loadings values GWPC 2.

For each cluster, we calculated the average values of factor loadings, which helped assess the specificity of the respective clusters (Fig. 10). For clusters 1 and 3 attenuation during the studied period is characteristic, while for clusters 2 and 4 a fading amplitude was observed in the middle of the research period. In the spatial aspect, cluster 4 occupies the west of the research area. Clusters 1, 2 and 3 are disruptive, so cluster 1 is mainly located in the center, cluster 2 – in the east, and cluster 3 – in the southwest of the research region (Fig. 11).

Principal component 1 (PCA 1) explains the largest part of soybean yield variability (33.5%). It is characterized by oscillatory dynamics with a period of 5 years and has the nature of an irregular component. Principal component 2 (PCA 2) has the amplitude of oscillation of 8–10 years. The principal components are spatially heterogeneous and divide the territory of Ukraine into 4 zones, which are characterized by different sensitivity of soybean yield to environmental factors. Such territorial clusters can be defined as agro-ecological zones, since an agro-ecological zone is an area with a similar course of ecological processes (Sivakumar & Valentin, 1997). Cosequenly, agro-ecological zoning refers to the division of an area (of land) into smaller units, which have similar characteristics related to land suitability potential production and environmental impact (Patel, 2003). The crop yield is a functional indicator of complex relations between plants and their environment (Anderson et al., 2013). Therefore, applying the yield as a basic indicator for agro-ecological zoning is entirely justified.

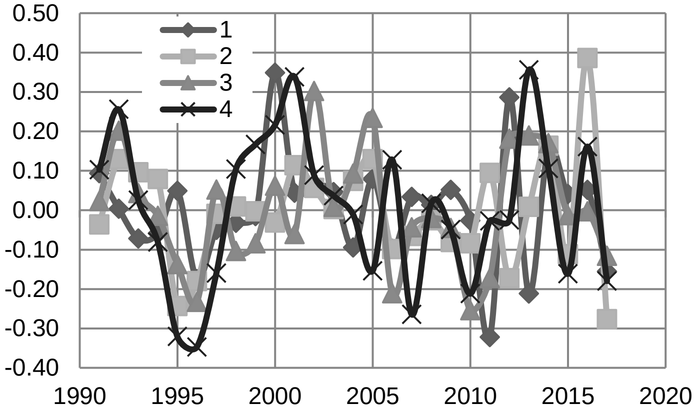


Figure 10. The average values of factor loadings of GWPC 2 for clusters 1–4.

Application of the principal components analysis of the yield dynamics is based on the assumptions that the origin of the relationships within the entire investigated area is homogeneous. Geographically weighted principal components analysis allows us to investigate local patterns of soybean yield dynamics (Patel, 2003). Local models have greater explanatory power than the total model, which is quite natural because the consideration of local specifics allows the more objective reflection of reality (Kumar et al., 2012). Nevertheless, the application of this approach causes certain methodological difficulties for meaningful interpretation. The most common technique of mapping 'winning' variables is not suitable in the case of time series analysis.

Consequently, based on the approximate types of local cycles, clusters were established for each principal component, and instead of displaying 'winning' variables, we applied the mapping of the established clusters. However, this approach has some advantages. Firstly, the ecologically homogeneous zones obtained by our approach (Figs 8, 11) are more compact than the ones that are established using 'winning variables' (Fig. 5). This result was obtained because in the clusters formation, the dominant role is played by the factors of a regular nature, and the random factors are filtered out during the analysis procedure (Zhukov et al., 2018). In fact, 'winning variables' are the result of a predominantly random choice from some lists of important information variables. Therefore, both approaches give a similar picture in general, but the proposed algorithm is less sensitive to random factors. Secondly, the proposed algorithm provides an opportunity to give a meaningful interpretation of the obtained clusters by studying the dynamics characteristics of each cluster in time. In the 'winning variables' approach, the variable itself is a marker of the corresponding spatially homogeneous territory (Kunah et al., 2018). Nevertheless, such an instrument is acceptable when qualitatively diverse variables are used, each of them can be measured in the next period, and thus applied to forecast the phenomenon under study. Among the time series variables, there are no 'more important' or 'less important' years. Besides, all of these variables are in retrospective and could not be re-measured. The patterns based on the cyclic frequency of processes are applied for forecasting. Such features can be set for the selected clusters. Results of the present work reveal that the GWPCA can be used for agro-ecological zoning.

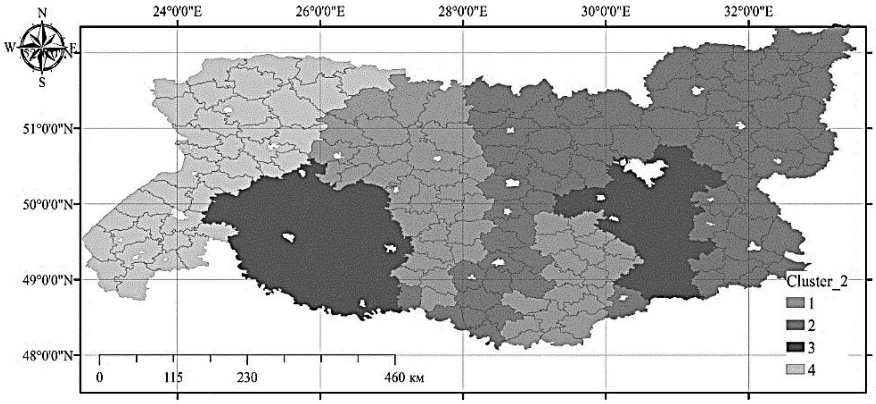


Figure 11. Spatial location of the clusters obtained based on the GWPC 2 loadings.

Consequently, agro-ecological zoning was performed with regard to the uniformity of dynamics of an agricultural area production potential. This approach is fundamentally different from that of zoning based on the total yield of crops (Lazarenko, 1995). A classification based on yields is justified for systems that are in a state close to the steady-state. According to the global climate changes and transformation of the environmental regimes, this approach is unacceptable. The agro-ecological zones proposed in the given research did not differ in the overall level of productivity of soybean during the study period. Features of these zones lie in the values of the principal components and reflect the nature of the relationship between different spatial units. Spatial distribution of the principal components indicates a continual pattern, but their overlapping allowed to determine spatially discrete units, which we identified as agroecological zones. Each zone is characterized by a certain character and dynamics of production capacity and has an invariant pattern of response to varying climatic, environmental, and agroeconomic factors.

CONCLUSIONS

Our study confirmed the hypothesis that within the studied territory there are zones with specific patterns of the temporal dynamics of soybean yield, which are uniform within each area but qualitatively different between zones. The principal components analysis of the regression models' residues of the time trend enabled us to establish 4 principal components, which together explain up to 58% of the variation in the regional soybean yield. Four spatially determined processes influence the yield of soybeans and have the oscillatory dynamics of different periodicity. The oscillating phenomena are of an ecological nature. Geographically weighted principal component analysis revealed spatial units with similar oscillatory component of soybean yield variation. The territorial clusters within which the temporal dynamics of soybean yield is identical can be considered as agro-ecological zones for soybean cultivation. Further study of the nature of the principal components will be the objective of our subsequent studies, as well as the impact of the climate change on the crop yield variability.

REFERENCES

Aggarwal, P.K. 1991. Agro-ecological zoning using crop growth simulation models: characterization of wheat environments of India. In: *Proceedings of the International Symposium on Systems Approaches for Agricultural Development*, Bangkok, Thailand, pp. 2–6.

Anderson, M.C., Cammalleri, C., Hain, C.R., Otkin, J., Zhan, X.W. & Kustas, W. 2013. Using a diagnostic soil-plant-atmosphere model for monitoring drought at field to continental scales. In: *Four decades of progress in monitoring and modeling of processes in the soil-plant-atmosphere system: applications and challenges*, 19, pp. 46–57.

Anselin, L., Ibnu, S. & Youngihn, Kh. 2005. GeoDa: An Introduction to Spatial Data Analysis. *Geographical Analysis* **38** (1), 5–22. doi: 10.1111/j.0016-7363.2005.00671.x

Comber, A.J, Harris, P. & Tsutsumida, N. 2016. Improving land cover classification using input variables derived from a geographically weighted principal components analysis. *ISPRS Journal of Photogrammetry and Remote Sensing* **119**, 347–360. doi: 10.1016/j.isprsjprs.2016.06.014.

Dinno, A. 2012. *paran: Horn's Test of Principal Components/Factors*. R package version 1.5.1. Available at: https://CRAN.R-project.org/package=paran

Gollini, I., Lu, B., Charlton, M., Brunsdon, Ch. & Harris, P. 2013. GWmodel: An R Package for Exploring Spatial Heterogeneity Using Geographically Weighted Models. *Journal of Statistical Software* **63**(17), 1–52.

FAO. 1976. A framework for land evaluation. Food and Agricultural Organisation, Soils Bulletin **32**, Rome, Italy.

FAO. 1996. Guidelines: Agro-ecological zoning. Food and Agricultural Organisation, Soils Bulletin, Rome, Italy.

Fotheringham, A.S., Brunsdon, C. & Charlton, M. 2002. *Geographically Weighted Regression: the Analysis of Spatially Varying Relationships*. John Wiley & Sons, Chichester, 284 pp.

Harris, P., Brunsdon, C. & Charlton, M. 2011. Geographically Weighted Principal Components Analysis. *International Journal of Geographical Information Science* **25**(10), 1717–1736. doi: https://doi.org/10.1080/13658816.2011.554838

Harris, P., Clarke, A, Juggins, S, Brunsdon, C. & Charlton, M. 2015. Enhancements to a Geographically Weighted Principal Component Analysis in the Context of an Application to an Environmental Data Set. *Geographical analysis* **47**(2), 146–172. doi: 10.1111/gean.12048.

Hengl, T., Mendes de Jesus, J., Heuvelink, G.B.M., Ruiperez Gonzalez, M., Kilibarda, M. & Blagotić, A. 2017. SoilGrids250m: Global gridded soil information based on machine learning. *PLoS ONE* **12**(2), e0169748. doi: https://doi.org/10.1371/journal.pone.0169748

Horn, J.L. 1965. A rationale and a test for the number of factors in factor analysis. *Psychometrika* **30**, 179–185. doi: 10.1007/BF02289447

Iqbal, J., Thomasson, J.A., Jenkins, J.N., Owens, P.R. & Whisler, F.D. 2005. Spatial variability analysis of soil physical properties of alluvial soils. *Soil Science Society America journal* **69**(4), 1338–1350. doi: 10.2136/sssaj2004.0154

Kaiser, H.F. 1974. An Index of Factorial Simplicity. *Psychometrika* **39**(1), 31–36.

Kaspari, M. & Yanoviak, S. 2009. Biogeochemistry and the Structure of Tropical Brown Food Webs. *Ecology* **90**, 3342–3351.

Kukal, M.S. & Irmak, S. 2018. Climate-Driven Crop Yield and Yield Variability and Climate Change Impacts on the U.S. Great Plains Agricultural Production. *Scientific Reports* **8**, 3450. doi:10.1038/s41598-018-21848-2

Kumar, S., Lal, R. & Lloyd, C.D. 2012. Assessing spatial variability in soil characteristics with geographically weighted principal components analysis. *Computational Geosciences* **16**(3), 827–835. doi 10.1007/s10596-012-9290-6

Kunah, O., Pakhomov, O., Zymaroieva, A., Demchuk, N., Skupskyi, R., Bezuhla, L. & Vladyka, Y. 2018. Agroeconomical and agroecological aspects of the rye (*Secale cereale* L.) yelds spatial variation within Polesia and Foreststeppe zones of Ukraine: the useage of the geographically weighted principal components analysis. *Biosystems Diversity* **26**(4), 276–285. doi: https://doi.org/10.15421/011842

Lazarenko, P.I. 1995. Ecological and biological bases of agricultural zoning areas (Dnipropetrovsk region as an example). Kyiv, 476 pp. (in Ukrainian).

Legendre, P. & Gallagher, E. 2001. Ecological Meaningful Transformations for Ordination of Species Data. *Oecologia* **129,** 271–280.

Li, Z., Cheng, J. & Wu, Q. 2015. Analyzing regional economic development patterns in a fast developing province of China through geographically weighted principal components analysis. *Letters in Spatial and Resource Sciences* **9**(3), 233–245.

Liu, J., Pattey E. & Jégo, G. 2012. Assessment of Vegetation Indices for Regional Crop Green LAI Estimation from Landsat Images over Multiple Growing Seasons. *Remote Sensing of Environment* **123**, 347–358. doi:10.1016/j.rse.2012.04.002

Lloyd, C.D. 2010. Analysing population characteristics using geographically weighted principal components analysis: a case study of Northern Ireland in 2001. *Comput. Environ. Urban.* **34**(5), 389–399.

Maier, M.J. 2015. Companion Package to the Book ``R: Einführung durchangewandte Statistik"_. R package version 0.9.3, URL: http://CRAN.R-project.org/package=REdaS

Moran, P.A.P. 1950. Notes on continuous stochastic phenomena. *Biometrika.* **37**(1/2), 17–23. doi: 10.2307/2332142

Patel, N.R. 2003. Remote sensing and GIS application in agro-ecological zoning. Satellite Remote Sensing and GIS Applications in Agricultural Meteorology. In: *Proceedings of a Training Workshop*, (7–11 July), Dehra Dun, India, pp. 213–233.

Pearson, K. 1901. On lines and planes of closest fit to systems of points in space. *Phil. Mag.* **2**(7–12), 559–572.

R Core Team. 2017. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available at: https://www.R-project.org/.

Ruiz-Vega, J. 1984. Soybean phenology and yield as influenced by environmental and management factors. *Retrospective Theses and Dissertations*, 8213. Available at: https://lib.dr.iastate.edu/rtd/8213

Sivakumar, M.V.K. & Valentin, C. 1997. Agroecological zones and the assessment of crop production potential. *Phil. Trans. R. Soc. Lond*. **352**(1356), 907–916.

Suriadikusumah, A. & Herdiansyah, D.G. 2014. Study on land resources based on agro-ecological zones in Bandung district, West Java -Indonesia. *International Journal of Applied Science and Technology* **4**(4), 212–220.

Zhukov, O.V., Pelina, T.O., Demchuk, O.M., Demchuk, N.I. & Koberniuk, S.O. 2018. Agroecological and agroeconomic aspects of the grain and grain legumes (pulses) yield dynamic within the Dnipropetrovsk region (period 1966–2016). *Biosystems Diversity* **26**(2), 3–10.

INSTRUCTIONS TO AUTHORS

Papers must be in English (British spelling). English will be revised by a proofreader, but authors are strongly urged to have their manuscripts reviewed linguistically prior to submission. Contributions should be sent electronically. Papers are considered by referees before acceptance. The manuscript should follow the instructions below.

Structure: Title, Authors (initials & surname; an asterisk indicates the corresponding author), Authors' affiliation with postal address (each on a separate line) and e-mail of the corresponding author, Abstract (up to 250 words), Key words (not repeating words in the title), Introduction, Materials and methods, Results and discussion, Conclusions, Acknowledgements (optional), References.

Layout, page size and font

- Use preferably the latest version of **Microsoft Word**, doc., docx. format.
- Set page size to **ISO B5 (17.6 x 25 cm)**, all **margins at 2 cm**. All text, tables, and figures must fit within the text margins.
- Use single line spacing and **justify the text**. Do not use page numbering. Use **indent 0.8 cm** (do not use tab or spaces instead).
- Use font Times New Roman, point size for the title of article **14 (Bold)**, author's names 12, core text 11; Abstract, Key words, Acknowledgements, References, tables, and figure captions 10.
- Use *italics* for Latin biological names, mathematical variables and statistical terms.
- Use single ('…') instead of double quotation marks ("…").

Tables

- All tables must be referred to in the text (Table 1; Tables 1, 3; Tables 2–3).
- Use font Times New Roman, regular, 10 pt. Insert tables by Word's 'Insert' menu.
- Do not use vertical lines as dividers; only horizontal lines (1/2 pt) are allowed. Primary column and row headings should start with an initial capital.

Figures

- All figures must be referred to in the text (Fig. 1; Fig. 1 A; Figs 1, 3; Figs 1–3). Use only black and white or greyscale for figures. Avoid 3D charts, background shading, gridlines and excessive symbols. Use font **Arial, 10 pt** within the figures. Make sure that thickness of the lines is greater than 0.3 pt.
- Do not put caption in the frame of the figure.
- The preferred graphic format is Excel object; for diagrams and charts EPS; for half-tones please use TIFF. MS Office files are also acceptable. Please include these files in your submission.
- Check and double-check spelling in figures and graphs. Proof-readers may not be able to change mistakes in a different program.

References

- **Within the text**

In case of two authors, use '&', if more than two authors, provide first author 'et al.':

Smith & Jones (1996); (Smith & Jones, 1996);

Brown et al. (1997); (Brown et al., 1997)

When referring to more than one publication, arrange them by following keys: 1. year of publication (ascending), 2. alphabetical order for the same year of publication:

(Smith & Jones, 1996; Brown et al., 1997; Adams, 1998; Smith, 1998)

- **For whole books**

Name(s) and initials of the author(s). Year of publication. *Title of the book* (*in italics*). Publisher, place of publication, number of pages.

Shiyatov, S.G. 1986. *Dendrochronology of the upper timberline in the Urals*. Nauka, Moscow, 350 pp. (in Russian).

- **For articles in a journal**

Name(s) and initials of the author(s). Year of publication. Title of the article. *Abbreviated journal title* (*in italic*) volume (in bold), page numbers.

Titles of papers published in languages other than English, should be replaced by an English translation, with an explanatory note at the end, e.g., (in Russian, English abstr.).

Karube, I. & Tamiyra, M.Y. 1987. Biosensors for environmental control. *Pure Appl. Chem.* **59**, 545–554.

Frey, R. 1958. Zur Kenntnis der Diptera brachycera p.p. der Kapverdischen Inseln. *Commentat.Biol.* **18**(4), 1–61.

Danielyan, S.G. & Nabaldiyan, K.M. 1971. The causal agents of meloids in bees. *Veterinariya* **8**, 64–65 (in Russian).

- **For articles in collections:**

Name(s) and initials of the author(s). Year of publication. Title of the article. Name(s) and initials of the editor(s) (preceded by In:) *Title of the collection (in italics)*, publisher, place of publication, page numbers.

Yurtsev, B.A., Tolmachev, A.I. & Rebristaya, O.V. 1978. The floristic delimitation and subdivisions of the Arctic. In: Yurtsev, B. A. (ed.) *The Arctic Floristic Region*. Nauka, Leningrad, pp. 9–104 (in Russian).

- **For conference proceedings:**

Name(s) and initials of the author(s). Year of publication. Name(s) and initials of the editor(s) (preceded by In:) *Proceedings name (in italics)*, publisher, place of publishing, page numbers.

Ritchie, M.E. & Olff, H. 1999. Herbivore diversity and plant dynamics: compensatory and additive effects. In: Olff, H., Brown, V.K. & Drent R.H. (eds) *Herbivores between plants and predators. Proc. Int. Conf. The 38th Symposium of the British Ecological Society*, Blackwell Science, Oxford, UK, pp. 175–204.

....

Please note

- Use '.' (not ',') for decimal point: 0.6 ± 0.2; Use ',' for thousands – 1,230.4;
- Use '–' (not '-') and without space: pp. 27–36, 1998–2000, 4–6 min, 3–5 kg
- With spaces: 5 h, 5 kg, 5 m, 5 °C, C : D = 0.6 ± 0.2; p < 0.001
- Without space: 55°, 5% (not 55 °, 5 %)
- Use 'kg ha^{-1}' (not 'kg/ha');
- Use degree sign ' ° ' : 5 °C (not 5 ^{O}C).