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Theoretical research into the power and energy performance of agricultural tractors

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Abstract. The widespread use of a great number of different types and manufacturer brands of tractors in agricultural use raises several important questions. These all concern the implementation of the criteria which may be involved in making the right choice in regard to a particular power unit that is capable of delivering the required result during the subsequent course of that unit's service life. Even more importantly, the result should be an economically sound one. Despite the fact that tractor theory offers a sufficient number of scientifically grounded criteria that characterise agricultural power units with respect to their particular properties, the engine power rating remains the most widely used and decisive figure – and the factor which defines the ultimate choice of power unit. Meanwhile, the traction properties of tractors, especially in case of wheeled tractors, should be of prime importance as these properties determine the maximum measures that can be taken in relation to efficiency levels in power units as parts of various unitised agricultural machines. Currently, in most areas around the world, the traction and energy performance of wheeled tractors is determined using the same common method, one which is based upon the tractor's power balance. But when taking into account the ever-increasing requirements for protecting the soil, the aforementioned method needs corresponding upgrading with respect to the destructive effect of the wheeled running gear of agricultural tractors on the soil's structure. The aim of this study is to develop a new method of determining the minimum required engine power rating for an agricultural wheeled tractor, as well as its operating mass and energy saturation rate when considering the linear type of dependence for its running gear slipping due to the tractive force being generated. The research utilises standard tractor theory and numerical computation methods. The completed study resulted in several updated and new analytical dependences, all of which can be used to define the tractive power of a wheeled tractor, taking into account the linear type of relationship between wheel slip, operating mass, and energy saturation rate. The data that is obtained through computational methods show that the classification of various wheeled tractors with regard to their traction or their traction and power category using the new method will subsequently allow more accurate calculations to be effected when it comes to unitising various agricultural machines, which should help to ensure an improvement in their overall performance levels.

Key words: tractor (agricultural), engine power, drawbar pull force, wheel slip, operating mass, energy saturation rate.

INTRODUCTION

The large number of different types and brands of tractors which are being widely employed in today's agricultural work raises several questions when it comes to the implementation of the criteria that permit the correct choice when it comes to a particular power unit. This is highly important as, when it is applied to agricultural production, it will provide the required results in terms of operation and, even more importantly, an economically sound result (Kutzbach, 2000; Nadykto et al., 2015). Currently, the theory behind tractor usage offers a sufficient number of scientifically grounded criteria that characterise agricultural power units with respect to their particular properties. That said, one of these characteristic values is used most widely and, in the majority of cases, it decides and determines the choice of a particular tractor – this being its engine power rating. Meanwhile, traction properties, which also characterise tractors, especially wheeled tractors, should in many cases be of prime importance since they determine the maximum measures in relation to the efficiency levels that these tractors will provide as parts of various unitised agricultural machines (Zoz & Brixius, 1979; Serrano, 2007; Šmerda & Cupera, 2010; Turker et al., 2012). Presently, the traction and energy performance of wheeled tractors is determined using virtually the same method, one which is based upon the tractor's power balance. But when taking into account the ever-increasing requirements in relation to protecting the soil (Godwin, 2007), and its structure and fertility, the aforementioned method needs corresponding updating with respect to the intensive destruction caused to the soil's structure by the wheeled running gear of agricultural tractors.

A method has been proposed in various papers for determining a tractor's minimum required operating mass (Boikov et al., 1997; Nadykto, 2014a), this mass being W_{tr} (kg) and its engine power rating N_e, (kW), basing this on the analysis of general power balance in terms of the present day understanding of the latter (Kutkov, 2004). A characteristic feature of this method is the due consideration given to the non-linear dependence of the power unit's running gear slip rate δ on the drawbar pull P_{dp}, (N) which is delivered by it. (Wong & Huang, 2006; Tiwar et al., 2010; Monteiro et al., 2013; Cutini & Bisaglia, 2016).

It should be noted that such a point of view of the function $\delta = f(P_{dp})$ has been and still remains undisputed within general tractor theory (Zoz and Grisso, 2003; Kutkov, 2004; Gil-Sierra et al., 2007; Nastasoiu & Padureanu, 2012; Turker et al., 2012; Abraham et al., 2014; Simikič et al., 2014). The maximum slip rate δ of the running gear on a wheeled tractor is restricted in this case to the value that provides its highest traction and energy performance levels, which usually reaches between 22% and 24% (Guskov et al., 1988; Moitzi et al., 2014). At the same time, in order to ensure that the soil's structure remains intact, the slip of a wheeled power unit may not exceed a level between 9% and 15%, as stated in the paper by Nadykto (2014b), at least in the spring and summer campaign period. If we take into account the fact that the great majority of state-of-the-art wheeled tractors produced across the world are all-wheel drive vehicles, which inherently implies better holding properties, then we face the need to revise the currently established point of view when it comes to the behaviour of the dependence $\delta = f(P_{dp})$.

The development of a new method for determining the minimum required engine power rating for an agricultural wheeled tractor, as well as its operating mass and energy saturation rate, subject to the linear type of dependence for its running gear slippage rate on the generated tractive force.

MATERIALS AND METHODS

The study was conducted using tractor theory, along with appropriate software development and numerical computation.

Based on the general provisions that have been stated in a previous paper (Nadykto, 2014b), the slip rate δ for the running gear of a wheeled tractor can be analysed exclusively in its linear interpretation, specifically:

$$\delta = A\frac{P_{dp}}{W_{tr}g} + B, \tag{1}$$

where *A*, *B* are approximation constants for the tractor running gear slip process which is represented in the form of a straight line; *g* is free fall acceleration.

However, this approach changes the nature of the tractor propulsion efficiency. It turns out that under certain conditions, especially in practice, its maximum (optimum) value can be altogether unattainable (Bulgakov et al., 2015). At the same time, the currently effective scientific provisions for the tractor theory *r* and machine usage stipulate that the maximum productivity of a machine and tractor unit can be achieved at the maximum propulsion efficiency of the tractor. The productivity of a machine and tractor unit is in its turn conditioned by such rated values for the power unit as its operating mass and engine power rating. This implies that, under the linear form of dependence $\delta = f(P_{dp})$, the method of determining these principal parameters for the tractor will become totally different. An examination of the main points of the said method is the topic of this study.

As with the work carried out in a previous paper (Nadykto, 2014a), and taking into account the methodical approaches laid down in (Kutkov, 2004), the equation for the tractor's power balance has to be set up for the consequent theoretical analysis, retaining the four main components of the power balance N_e for the power unit, ie. the agricultural wheeled tractor, which will result in the following power balance equation:

$$N_e = N_{dr} + N_{tr} + N_{\delta} + N_{mr}, \tag{2}$$

where N_{dr} is the tractive power of the tractor itself; N_{tr}, N_{δ}, N_{mr} are the power rates that specify energy consumption by friction in the transmission, the slip of the running gear, and the rolling resistance of the power unit.

Each component in the formula (2) can be expressed as follows, in the form of a set of analytical dependences:

$$\begin{aligned} N_{dr} &= P_{dp} \cdot V = P_{dp.n} \cdot (1 + 3 \cdot V_x) \cdot V, \\ N_{tr} &= (1 - \eta_{tr}) \cdot N_e, \\ N_{mr} &= f \cdot W_{tr} \cdot g \cdot V, \\ N_{\delta} &= (f \cdot W_{tr} \cdot g + P_{dp}) \cdot \delta \cdot V. \end{aligned} \tag{3}$$

The following designations are assumed in the presented equations (3): V is the operating speed of the tractor as part of the particular machine and tractor unit; $P_{dp.n}$ is the rated drawbar pull of the tractor; V_x is the coefficient of variation in the power unit's traction load; η_{tr} is the efficiency coefficient in the tractor's transmission; and f is the rolling resistance coefficient of the power unit.

After substituting the dependences (1) and (3) into the equation (2) and making the respective transformations, the value N_e will be presented as follows:

$$N_e = \frac{W_{tr}^2 D_2 + W_{tr} D_1 + D_0}{W_{tr} D_3}, \tag{4}$$

where

$$D_0 = \frac{A\left[P_{dp.n}(1+3V_x)\right]^2 V}{g},$$

$$D_1 = P_{dp.n}(1+3V_x)V(1+B+fA),$$

$$D_2 = fgV(1+B), \qquad D_3 = \eta_{tr}.$$

RESULTS AND DISCUSSIONS

The optimum value for the tractor's operating mass can be established by means of solving the partial derivative $\frac{\partial N_e}{\partial W_{tr}} = 0$. This results in the following presentation of the tractor's operating mass:

$$W_{tr} = \frac{P_{dp.n}(1+3V_x)}{g}\sqrt{\frac{A}{f(1+B)}}. \tag{5}$$

After finding the tractor's operating mass from the formula (5), and substituting it into the expression (4), its minimum required engine power rating can be calculated.

To start off, it can be concluded from the analysis of the formula (5) that, when the tractor's coefficient of rolling resistance f increases, its operating mass W_{tr} should decrease. In effect, this is not the case and the reason is as follows. The rising coefficient f implies the deteriorating conditions of adhesion between the power unit's running gear and the soil. When the moisture content of the soil is normal, this effect can also take place because the soil itself is considerably loosened. This is why the tractor's coefficient for rolling resistance f is always lower on hard soil (for example, on stubble), than it is on tilled soil (for example, soil that has been prepared for planting).

The path of a tractor that is travelling with the same pulling force on hard soil as it is on loosened soil will, in the latter case, feature a greater degree of running gear slippage. Analytically speaking, this will be added to the picture through the respective values of the aforementioned approximation constants, A and B.

The results of the analysis of traction performance for a number of wheeled tractors indicates that the growth of the coefficient A has the greatest effect on the value W_{tr}, which is something that cannot be said about the growth of the coefficients f and especially B. Moreover, the rate of growth for the approximation constant A prevails to such an extent that, as a result, with the parameters $P_{dp.n}$ and V_x in the formula (5) remaining unaltered and the value of the tractor's rolling resistance coefficient f increasing, its operating mass W_{tr} will grow.

The necessary and most desirable level of reliability in the application of the formulae (4) and (5) for specific calculations can be achieved only in the case of a sufficient quantity of data being available with respect to the values of constants *A* and *B* for the linear approximation of slippage in the running gear of a wheeled tractor under the conditions of having to function on different types of cultivated land. So far, no one has managed to obtain any such traction performance data for tractors.

Nonetheless, despite this fact we will try and apply the formulae (4) and (5) for actual calculations. For this purpose, the values for the components of these expressions first have to be set. This applies to the upper limit for the operating travel speeds of machine and tractor units. In the course of practical operation, it has been revealed that the average value of that parameter for the majority of state-of-the-art tilling and sowing agricultural machine and tractor units is approximately equal to 9 km h^{-1}. In this case, the coefficient of the tractor's traction load variation can have a value that is between 12% and 18% (Guskov et al., 1988). Taking that into account, the value $V_x = 0.15$ will be assumed for the calculations.

Furthermore, as an example, consideration will be given to the wheeled power units in traction category three, which are fairly common on European farms. They have integrated design layouts, and locked wheel drives on the front and rear axles. According to data that has been obtained from the drawbar tests on a broken stubble field at a coefficient of rolling resistance of $f = 0.11$ and a maximum running gear slip of 12%, their average rated drawbar pull is $P_{dp.n} = 32$ kN. The coefficients for the approximation of the slippage process for these tractors in the form of a straight line are as follows: $A = 0.301$; $B = 0.001$. The transmission efficiency factor is $\eta_{tr.t} = 0.93$ (Kutkov, 2004).

The results of the computation for the formula (5) show that, with such basic data, the operating mass of a traction category three tractor should be 7.8 t. This is at least 0.3 t less than the actual operating mass of those power units that are produced by most of the manufacturers in Europe.

For a comparison, the authors of a past paper (Guskov et al., 1988) propose that the tractor's operating mass be estimated using the following formula:

$$W_{tr} = \frac{\Delta_{\lim} \cdot F_{kr.n}}{\left(\varphi_{dop} - f\right) g} \tag{6}$$

where $\Delta_{\lim}$ is a factor of the tractor's potential tractive effort overload. In this case $\Delta_{\lim} = 1 + 3V_x$; $F_{kr.n}$ is the rated tractive effort of the power unit, ie. it is equal to $P_{dp.n}$; φ_{dop}, the adhesion coefficient of the between the tractor's running gear and the soil which is acceptable under the existing agrotechnical conditions. The maximum value of this parameter as suggested by previous authors (Guskov et al., 1988), ie. $\varphi_{dop} = 0.75$, will be assumed.

With the following initial data: $V_x = 0.15$; $F_{kr.n} = P_{dp.n} = 32$ kN; $\varphi_{dop} = 0.75$ and $f = 0.11$, it follows from the formula (6) and the data that has been obtained by computation on a PC that the operating mass of a traction category three tractor has to be equal to 7.4 t. This is only 400 kg less than its value as obtained in our computation of the formula (5).

As regards engine power, the minimum rating here has to be almost 174 kW (ie. 237 hp), which follows from the calculations with the use of the formula (4). A detailed account of the method used in selecting the full engine power rating of the tractor is given in a previous paper (Nadykto, 2014a).

It should be stressed that, currently, this rating at its maximum value for traction category three tractors is equal to a mere 175 hp to 180 hp, which is 26% below the estimated level. It has been proven thanks to the operating practice employed by the majority of agricultural wheeled tractors in traction category three that it is the shortfall of their engine power that curbs the speed performance of these power units in state-of-the-art tillage and crop sowing. This has an adverse effect on both the productivity and the economic feasibility of the farm operations which they carry out.

Apart from the operating mass W_{tr} and engine power rating N_e, there is one more important design parameter for a tractor, which is its energy saturation rate E_{tr}. Analytically speaking, a wheeled tractor's energy saturation rate can be represented by the following expression:

$$E_{tr} = \frac{N_e}{W_{tr}} = \frac{W_{tr}^2 \cdot D_2 + W_{tr} \cdot D_1 + D_o}{W_{tr}^2 \cdot D_3}. \quad (7)$$

According to the formula (7), a wheeled tractor's energy saturation rate has a dimension of (kW t^{-1}). Recently, a number of authors have been considering this parameter as the ratio between the tractor's engine power rating and its operating weight (Kutkov, 2004; Rebrov & Samorodov, 2010). In that case the dimension is (kW $(kN)^{-1}$). It is easy to show that the latter dimension represents the translational velocity of the tractor, ie. in effect the dimension is (m s^{-1}).

Our opinion is that the dimension (kW t^{-1}) better reveals the essence of the energy saturation rate E_{tr}, by showing how much of the tractor's engine power N_e is accounted for as a unit of its mass. At the same time, the dimension of (m s^{-1}) provides little information since the translational velocity of the tractor as part of a particular machine and tractor unit can be limited not by the power unit's engine potential, but by the agronomical and/or other requirements.

It is emphasised in a study by Nadykto (2012) that a tractor with an energy saturation rate of 14–15 kW t^{-1} is a traction concept power unit; in the case of higher energy saturation rates the traction and power concept is applicable. The latter stipulates that designers of agricultural tractors have to develop a system that utilises via various parts of the machine and tractor unit that part of the engine power which cannot be utilised through the drawbar pull.

The calculations for the formula (7) have shown that in order for a tractor as part of a particular machine and tractor unit to be able to utilise a tractive effort of 32 kN at an operating speed of 9 km h^{-1} and the linear form of the dependence of its running gear slipping on the tractive force, the energy saturation rate for the tractor has to be at a level of 22.3 kW t^{-1}. With that figure in mind, the tractor becomes a power unit that fulfils the traction and power concept. Meanwhile, in practice the power units in the overwhelming majority of traction category three wheeled tractors still remain exponents of the traction concept, since their energy saturation rate E_{tr} remains within 16 kW t^{-1}.

As the parameter E_{tr} is determined by the ratio between the tractor's installed engine power and its operating mass, it remains constant over the whole service life of the power unit. Or at least over a time interval within which the value N_e remains constant.

When the tractor's traction load is variable (which turns out always to be the case), in practice its installed engine power cannot be utilised completely, as stated by Kutkov (2004). That implies that the tractor's energy saturation rate is a potential property and, as opposed to the statement by Rebrov & Samorodov (2010), it does not depend on the

mode of travel for the machine and tractor unit. It can only be changed by installing in the tractor an engine which has another power rating or by ballasting the power unit, or applying the first and second measures simultaneously. In terms of the compacting effect on the soil medium, the overall prospects of tractor ballasting are rather poor and this approach is undesirable even if the tractor is designed in accordance with the principles of traction and power. This problem is further examined in a study by Nadytko (2013).

CONCLUSIONS

When a certain level of drawbar pull ($P_{dp.n}$), in mode (V, V_x) and under the conditions (f, A, B) in the operation of a wheeled tractor are targeted, the formula (5) enables the operating mass E_{tr} to be determined, while the expression (4) stipulates the minimum required engine power rating N_e.

When operating within these specifications it is possible to specify the train of agricultural machines or implements that need to be unitised with a tractor that features such principal design parameters, subject to the linear type of slip rate variation which it may experience with the generated tractive force. The principles of selecting the composition of the machine and tractor unit for a particular rated drawbar pull of tractor are commonly known.

The tractor's energy saturation rate as the ratio between the installed engine power rating and the operating mass of the power unit is the criterion of its belonging either to the traction or the traction and power concepts, each of which feature their own system of unitising agricultural equipment. This criterion should be understood by the designers of any new mobile power units for operation in the agricultural industry.

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Developing a new design of wood chopper for grape vine and fruit tree pruning and the results of field testing

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Abstract. The problem of collecting and utilising the pruned canes of grape vines and branches and twigs from fruit trees that are left in vineyards and orchards all year round after the scheduled pruning of plantations in early spring is the topic of the day, and it is one that requires an effective solution. One of the ways in which the problem might be solved is the utilisation of pruning material as an organic fertiliser after it has been gathered, so that it is chopped, evenly spread in the inter-row spaces in the plantation, and ploughed into the soil, so that it decomposes there, and is digested. Meanwhile, the degree of pruned material disintegration and the level of quality shown in the work of spreading it across the area both have to ensure the complete decomposition of any such ploughed-under wood waste within one calendar year. The aim of this research project was to increase efficiency levels in chopping and spreading grape vine and fruit tree pruning material on the basis of the development of a new design of wood chopper and the results obtained in field testing this piece of equipment. The research uses engineering and design methods which are based on the theory of collecting from ground level and further transporting wood and plant materials, the theory behind cutting, crushing, and spreading, and also the methods used in experimental research, field testing, and the statistical analysis of test results. We have developed a new design arrangement for a wood chopper for grape vines and fruit trees, one which combines the mechanism for collecting slender, flexible waste wood pieces that are of a considerable length from the ground with a system that involves the transportation, chopping, shredding, and spreading over the soil surface of such materials. The prototype wood chopper design which was subsequently produced has been tested for several years in the laboratory and in field conditions and has delivered positive results. From the results of the field tests it has been found that, when using the aforementioned work process, a considerable reduction is achieved in terms of energy consumption and labour input in comparison with similar indicators for wood choppers that have been produced by recognised manufacturers. For example, the power demand for one metre of the machine's working width is just 15 kW, which is virtually two times less than the respective figure for a similar, recognised machine. The degree at which pruning material is collected from the ground is 95.4%, while the degree at which they disintegrate lengthwise is within a measurement of 10 cm, while the weight of the chopper is 1.5 times smaller than that of the similar machine used in the comparison studies. The use of these wood choppers provides an

opportunity to implement widely across the horticultural industry those innovative technologies that not only aim at reducing energy and labour consumption, but which also help substantially to cut down the demand for the input of mineral fertilisers, which improves the overall ecological characteristics of the natural environment.

Key words: vineyard, fruit tree, chopper, product design and development.

INTRODUCTION

Currently, in Ukraine, Moldova, Russia, Armenia, and other countries prunings from vines, as well as branches and twigs that have been pruned from fruit trees, are in the overwhelming majority of cases collected from the plantation's inter-row spaces, following which they are transported outside the planted area and are simply burned. This is especially customary in the case of pruning materials from vines, because the length of pruned canes can be either large or small (Ntalos & Grigoriou, 2002), while their diameter is always small and therefore it is not practical to use them further for any purpose other than burning. At the same time, this method of collection, transportation, and utilisation of pruned material not only consumes considerable amounts of energy and labour, but also results in additional levels of environmental pollution.

According to the results of our calculations, only in Crimea does the annual volume of unproductive incineration amount to about 180,000 tons of grape vine and fruit tree waste wood produced during the scheduled pruning of branches and canes in early spring. Undoubtedly, this causes significant damage to the environment (Calatrava & Franco, 2011; Gonçalves et al., 2011; Spinelli et al., 2014). Meanwhile, the calculations also show that the calorific capacity of waste wood in the form of pruned grape vine canes and fruit tree branches is close to $15.3\ 10^3$ MJ t^{-1}, which at the aforementioned volume is equivalent to burning down 112,500 tons of coal. At the same time, between 10 to 14 kg of nitrogen, between 6 to 8 kg of phosphorus, and between 12 to 15 kg of potassium are taken away from each hectare of grape vine and fruit tree plantation together with pruned canes and branches. In this manner, the accumulated chemical and energy potential of the annual gain in biomass effectively is lost.

The technology that enables pruned vine canes and fruit tree branches to be reduced into segments of a length of under 10 cm and then ploughed into the soil in plantation inter-row spaces (which is a task that is already carried out each year) – ie. the utilisation of pruning material that is chopped up and is later digested as a valid organic fertiliser – is capable of preventing the aforementioned losses and can also protect the environment. The pruned canes which were reduced to the indicated degree (ie. to sizes with a length of under 10 cm) will be fully digested into the soil and turned into an organic fertiliser within one calendar year.

The indispensable need to replenish the soil's resources in grape vine plantations with fertilisers is due to the fact that the grape vine as a monoculture is cultivated in the same place for thirty years or more. At the same time, those soil types which are appropriate for grape cultivation are generally rather lean when it comes to their nutrient content. They can easily be dry, stony soils with a humus content of only 1–2%. Therefore, the chopped mass of grape vine pruning material which is retained in the field and is ploughed into the soil will, after it has been digested and turned into a fertiliser,

contribute to the natural improvement of the soil's fertility and decrease the overall demand for the application of mineral fertilisers.

However, the possibility of using the chopped up and collected mass of grape vine pruning material for some other purpose is not excluded from consideration (Ntalos & Grigoriou, 2002; Benito et al., 2006; Youkhana & Idol, 2009; Calatrava & Franco, 2011; Rosua & Pasadas, 2012; Gomes-Munoz et al., 2016; Corona & Nicoletti, 2010). For example, such material can be used for the production of fuel bricks, which can be as important in energy crisis periods. That said, if we estimate the level of costs associated with this specific type of utilisation, then it becomes evident that the use of chopped up grape vine canes as an effective organic fertiliser is of a higher priority than the second method of utilisation for such material.

Currently, research into the rational use of grape vine pruning material is in progress, both within our country and abroad. Several production prototypes of machines have already been developed for collecting and chopping up pruned grape vine canes (Recchia et al., 2009; Spinelli & Picchi, 2010; Spinelli et al., 2010; Acampora et al., 2013; Managnotti et al., 2013) and then spreading them over the soil's surface. In this process, there is a pronounced trend towards developing dedicated pick-up wood choppers for grape vine and fruit tree pruning material. And for these machines, towed, semi-mounted, and fully mounted versions of the design layout are developed. When analysing the process and design features of agricultural implements of this type where they already exist in the world, it can be seen that pick-up wood choppers, as a rule, collect pruned grape vine canes or fruit tree branches and twigs from the ground with the use of a separate pick-up attachment, and then transfer the material to the machine's adjacently-located wood chopping equipment. There are two types of pick-up attachments: passive, meaning the involvement of rake type equipment, and active, meaning rotor type equipment, in the form of drums with fingers (rods) or belts with fingers. The latter are designed as belt conveyors, which feature fingers for collecting and lifting pruned waste wood, attached to the belt with the use of strips.

The chopping tools can be divided into the following main types according to the method used in disintegrating wood and plant material: cutting, chopping, sawing, and crushing. When it comes to their design, almost all wood choppers are made in the form of fast-rotating drums to which are attached some form of cutting or crushing implement (in the form of knives or hammers or similar). Depending on the form of implement being used, the wood chopper drums are accordingly equipped with shear bars, decks, or concaves. By far the majority of grape vine wood chopper designs have been developed in France, but their embodiments are rather complex and energy-intensive machines, which are very highly-priced and involve considerable cost in terms of their operation (Spinelli et al., 2014).

The detailed analysis of the existing designs of wood chopper for grape vine and fruit tree pruning material has shown that, in general, these machines feature the following essential drawbacks: high energy intensity involved in the process, the non-uniformity of segment sizing in the final product with a high content of segments that have been chopped up insufficiently, low reliability levels, and a complexity of operation. Due to the diverse composition of the raw material that is being collected from the ground (apart from wood and plant pruning material) – specifically: stones, metal, or concrete chips from the posts – intensive wear and tear occurs and frequent breakdowns are recorded for collection tools and, especially, chopping tools.

The aim of the research was improving the efficiency of chopping and spreading grape vine and fruit tree pruning material on the basis of the development of a new design of wood chopper and the results of its field testing.

MATERIALS AND METHODS

Used within the research are those methods of engineering and design that have been based on theories which are related to collecting wood and plant material from the soil's surface and further transporting such material, plus theories which are related to the cutting, crushing, and spreading of such material, and also the methods being used in experimental research, field testing, and the statistical analysis of test results.

RESULTS AND DISCUSSIONS

On the basis of the exploratory theoretical and experimental studies that have been carried out, including associated engineering and design work, a breadboard model of the new grape vine and fruit tree pruning wood chopper has been developed. As part of the development process, substantial modifications were made to the wood chopper design, and these were applied with regard to ensuring efficient operation in conventional orchards, trellis system orchards, and various types of vineyard.

The industrially-produced wood chopper prototypes have undergone laboratory and field tests. The preliminary results of the tests provide evidence of achieving the following points:

– increased quality in terms of pruned wood chopping and spreading over the field;
– improvements in the ecological state of the natural environment;
– a reduced demand for fertilisation;
– significant lowering of energy consumption levels in comparison with similar machines that are made by foreign manufacturers.

Moreover, the chopper falls into the category of innovative products (Pahl et al., 2007), with these being in line with the priority trends in the mechanisation of labour and energy intensive processes in horticulture and viticulture in many countries around the world.

Over the next few paragraphs, we are going to examine in detail the design and performance characteristics of the grape vine and fruit tree waste wood chopping machine that we have developed.

The grape vine and fruit tree waste wood chopper is intended primarily for collecting, chopping, and spreading vine canes in vineyard plantation inter-row spaces and wood that is pruned from fruit trees at an inter-row spacing of at least 2.5 m. The application of this wood chopper with other inter-row spacing values is possible, but in such cases the achievement of high operation and performance figures is not guaranteed.

The wood chopper allows for the collection and chopping of pruned grape vine canes of any length with a diameter of up to 35 mm (this dimension will also be applied in cases in which the wood chopper is being used for pruned branches and twigs from fruit trees), with the output being reduced to small segments with a length of under 10 cm, while the machine itself can operate on level plots in orchards and vineyards, and also on slopes with a gradient of up to five degrees, and on all types of soil, including stony ground (ie. soils which contain stones that have a diameter of up to 80 mm). The

design of the pick-up system precludes the entrainment of stones and other non-vegetable material from the soil's surface which has a dimension that is greater than that already mentioned. The pruning material wood chopper is aggregated with a wheeled tractor.

The design and process setup for the new grape vine and fruit tree waste wood chopper is presented in Fig. 1. Fig. 2 shows the chopping unit (top view).

The major elements of the new pick-up wood chopper design are: frame (1), with a front section onto which the pruned wood collection unit (2) is mounted; plus tool drive components including: a hydraulic motor (7) which drives the collection unit (2), drive gear (4) (driven by the power take-off shaft of the aggregating tractor), which is installed on the central part of the frame (1), intermediate gear (5) and belt transmission (6), the chopping unit with blades (3) and a specially designed upper casing, free-wheel clutch (8), and supporting gauge wheels (9) (Fig. 1).

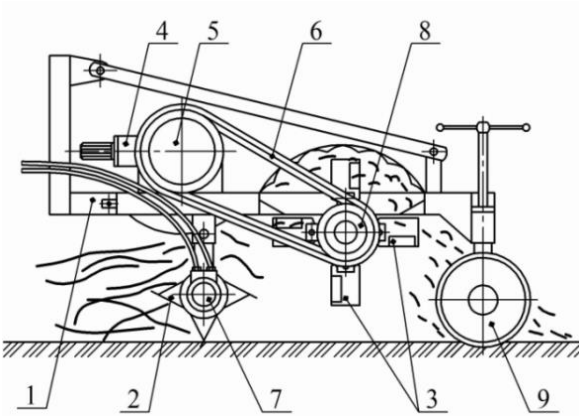

Figure 1. The design and process setup of the grape vine and fruit tree waste wood chopper (sectional side view): 1 – frame; 2 – pruned wood pick-up unit; 3 – chopping unit blades; 4 – drive gear; 5 – intermediate gear; 6 – belt transmission; 7 – pick-up unit drive hydraulic motor; 8 – free-wheel clutch for chopping unit drive; 9 – gauge wheel with height adjustment mechanism.

The frame (1) on the chopper is a welded assembly that is fabricated from rectangular tubes and channel beams. The front of the frame features a rigidly-fixed standard three-point linkage for hinging to the aggregating tractor.

The collecting unit (2) consists of a tubular shaft with welded rods which are installed on the lower part of the frame (1) with the use of telescopic struts, which allow adjustments to be made in the vertical position of the collecting unit (2) in steps to a total of 200 mm by repositioning the locking pins.

The chopper drum with chopping blades (3) consists of a tubular shaft, to which clevis lugs made from angled sections are welded. In the clevis lugs, two types of chopping blade are installed so that they pivot: straight blades and L-shaped blades. The chopper drum shaft rotates on two bearing supports which are attached to the frame with the use of stud bolts and collar clamps (Fig. 2).

The actuation of the chopper drum is provided by the aggregating tractor's PTO shaft (at 540 rpm) which drives the step-up gear (4), and then the intermediate gear (5), the belt transmission (6), and the free-wheel clutch (8). The last item is mounted directly onto the shank of the chopper drum. At the top, the chopper drum is covered with a special casing.

The described chopper design utilises a single-stage step-up bevel gear (with four positions) that increases the speed of rotation at a gear ratio of 2.4.

The intermediate gear (5) consists of the shaft (which connects the central part of the frame (1) with its side end), which is installed on two bearing supports and is connected to the gear unit (4) by the use of a bush roller coupling. The other end of the shaft of the intermediate gear (5) features the attached greater pulley for the V-belt transmission (6). The intermediate gear (5) and belt transmission (6) are covered with protection casings.

The gear unit (4) and intermediate gear (5) are mounted onto the frame (1) by the use of a special plate to which they are bolted. The plate is also fastened to the frame (1) using bolts that pass through elongated holes, which allow the plate to be shifted on the frame (1) in order to adjust the tension in the belts at the belt transmission (6). In order to be able to carry out this adjustment, the two tension bolts connecting the front part of the frame (1) and the aforementioned special plate are used.

The pick-up unit (2) is driven by the hydraulic motor (7) which is mounted onto the collecting unit's support bracket and is connected by pipelines to the aggregating tractor's hydraulic system. Two supporting gauge wheels (9) provide for the adjustment of the positioning of the frame (1) in relation to the soil's surface, depending on the height of the windrow of pruned grape vine canes or fruit tree branches and twigs.

Fig. 3 shows the general view of the new grape vine and fruit tree pruning material wood chopper, which was manufactured and tested first in a laboratory, and then also in field conditions (rear view).

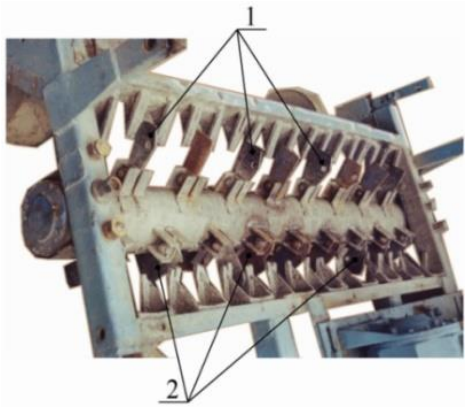

Figure 2. Chopping unit: 1 – straight blades; 2 – L-shaped blades.

Figure 3. Experimental model of the wood chopper.

The work process that can be managed by the collecting unit of the new design is now described further. The aggregating wheeled tractor moves progressively through the inter-row spaces in the grape vine or fruit tree plantation at a pre-set travel speed. The wood chopper, mounted on the aggregating tractor's rear hitch, picks up pruned wood that lies on the soil's surface in the inter-row spaces, using the collecting unit (2) to achieve this and then transferring the material into the wood chopper's upper section, into the operating zone of the chopper drum. The chopper drum entrains the pruning material with its blades (3) and draws it through the casing that covers the drum from above. The chopper drum's upper casing has a design which ensures that the recesses which is arranged on its inner surface plays the role of shearing blades. As a result of such an arrangement, the straight blades (3) at once efficiently cuts up pruned material into smaller segments. Furthermore, the L-shaped blades (3) also entrain the waste wood and crushes it to the required degree (to a length of under 10 cm). In this process, the ratio between the wood chopper's drum revolution rate and the wood chopper collecting travel rate is selected so that it ensures an adequate chopping quality and high levels of productivity.

During the machine's progress forwards along its path, the rear wheels of the aggregating tractor and the gauge wheels (9) of the wood chopper ensures that soil surface irregularities in the inter-row spaces grape vine and fruit tree plantation do not result in any irregularities in terms of collecting up material. The wood chopper is placed in the horizontal position at the required height by using the mechanism in the gauge wheels (9). At the same time, the shaft of the waste wood collecting unit (2) has been designed so that its rods can enter the top soil easily, almost entirely without entraining and lifting up soil particles. Also, the design allows the elevation of the collecting unit (2) to be adjusted with the use of the telescopic struts and lock pins. After chopping up pruned grape vine canes or fruit tree waste wood, the blades (3) in the chopper drum spread the resultant material evenly over the soil's surface.

In the period between 2011 and 2015, annual field tests of the new wood chopper design were carried out in three vineyards in the Crimea, each being located in a different area – on a plain, in the foothills, and in the mountains. During these tests, the existing standard test procedures were used, measurements were recorded and processed, and the wood chopper in our design was compared to the TRP-175 grape vine cane shredder (KUHN).

After a statistical analysis had been carried out on the results of the five-year field testing process, the designers were able to arrive at finalised results for the principal processes, performance, and operational indicators which are characteristic of the operation of the compared models. The data obtained in these long-term tests are presented in Table 1.

Table 1. The comparative characteristics of process, performance, and operational features of the new design of wood chopper and a similar machine

Pos. no	Technical and economic index (description and measurement unit)	TRP-175 Shredder (KUHN)	Experimental model of chopper
1	Production rate per hour of productive time (ha h^{-1})	1.43	1.43
2	Operational travel speed (m s^{-1})	1.24	1.24
3	Effective width (mm)	1,750	1,500
4	Width of processed inter-row spaces, at least (m)		2.5
5	Overall dimensions (mm):		
	length	1,750	1,500
	width	2,160	1,860
	height	1,210	1,010
6	Weight (kg)	950	650
7	Diameter of collecting drum at rod ends (mm)	350	355
8	Drive and revolution rate of collecting drum (rpm)	belt transmission 350	hydraulic motor 350÷450
9	Type of wood chopper drum	hammers	blades
10	Diameter of wood chopper drum (mm)	465	485
11	Revolution rate of wood chopper drum (rpm)	1,960	2,150
12	Number of blades/hammers (pcs)	28	34
13	Power consumption (kW hp^{-1})	49/67	22.5/30.6
14	Pick-up ratio (%)	89	95.4
15	Average length of segments after disintegration (cm)	1.4	4.8

It can be seen from the data shown in the table that the weight of the experimental model of wood chopper is one and-a-half times less, and is equal to 650 kg versus the 950 kg of the TRP-175 shredder, while its power consumption per metre of working width is a mere 15 kW versus the 29 kW of the model being used as a comparison. The experimental wood chopper model shows a higher level of accuracy when it comes to collecting pruned materials from the soil's surface, ie. 95.4% versus the 89% of the TRP-175 shredder.

The degree to which collected grape vine pruning material is chopped up by the experimental chopper meets the agricultural requirements for the respective work process. The volume of chopped segments that have a length of 10 cm does not exceed 5% of the total mass of the chopped vine. Meanwhile, the average length of segments being outputted by the experimental chopper is 4.8 cm, which is greater than in the case of the compared TRP-175 shredder (1.4 cm). However, such minimal dimensions are not required for the chopped mass of waste wood, since agrochemical studies that have already been conducted have shown that chopped segments with a length of up to 5 cm, if ploughed under the soil, can be digested within one calendar year just as effectively as any smaller segments. At the same time, the high degree of grape vine shredding in the French shredder is achieved by positively pressing the chopped mass through the deck (screen) with a mesh size of no more than 2.5 cm^2. But it is just this type of shredding process that results in a two-fold increase in the power consumed by the drive of the chopping/shredding drum.

Fig. 4 shows an area in the inter-row space in a vineyard plantation after the spring pruning of the grape vines. In Fig. 5, the same area is shown after a run-through by our new design of wood chopper.

Figure 4. A section of the inter-row space in a grape vine plantation after spring pruning for vines, with the grape vine pruning material piled in the middle.

Figure 5. The same section of inter-row space in a grape vine plantation after the pruning material has been chopped up.

The results that we obtained from the completed field tests for the experimental model of wood chopper and the data from literature about similar results of tests with wood choppers/shredders that have been produced by other foreign manufacturers show that our design of experimental wood chopper model consumes between 1.5 to 2.0 times less metal per metre of working width, while providing a rather high level of chopping quality. According to our calculations, the annual economic benefit from the implementation of the described experimental chopper in 2015 in Ukraine had amounted to more than 2,500 dollars. At the same time, the implementation of the mechanised technology for collecting, chopping, and spreading the chopped mass over the soil's surface reduces the demand for fertilisation by 25%.

Following the completion of calculations, we have found that the annual demand for such pruning material wood choppers in Ukraine is at least 400 units, while in Russia about 600 units would be required, and in Armenia (for chopping fruit tree pruning material) at least 150 units would be required at an annual workload of 270 ha per chopper. In the case of the mass use of such wood choppers in the horticultural industry, the indicated figures should be multiplied by a factor of ten. Therefore, annual production of the new machines has to be around 1,000 units with a service life of at least six years. According to the preliminary calculations, the cost of one chopper at 2013's rates was 5,000 dollars.

CONCLUSIONS

1. A new wood chopper has been developed for grape vine and fruit tree pruning material . The machine has a simple and robust design, which allows the work process to be carried out when it comes to mechanised collection, transportation, and chopping up pruning material and spreading the chopped mass over the soil's surface at a high level of quality and with improved efficiency.

2. The industrially-produced prototype of the new wood chopper has, for several years, passed laboratory and field tests and has delivered exceptional results with respect to the quality and efficiency of its operation.

3. The results of the field tests show that a considerable reduction in energy and labour consumption is achieved in the described waste wood disintegration work process when compared to similar indicators for wood choppers that have been produced by recognised manufacturers. The power consumption rates for the proposed wood chopper is 15 kW per single metre of working width, which is virtually two times less than in the case of the other, similar machine. The level of completeness in terms of collecting pruning material from the soil's surface exceeds 95%, while the degree of disintegration lengthwise remains within 10 cm and is 4.8 cm on average. The weight of the improved wood chopper is 1.5 times less than the weight of a similar machine with which it was compared.

4. The use of the new wood chopper will facilitate the implementation of innovative technologies in horticulture and viticulture, aiming not only at a reduction in energy and labour consumption, but also at the substantial lowering of the demand for mineral fertilisers, which will significantly improve the ecological indices of the natural environment.

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Phytochemical diversity of fennel landraces from various growth types and origins

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Abstract. The presence of certain secondary metabolites in fennel essential oil is the cause of its pharmacological and flavoring properties. In this study phytochemical diversity including essential oil content and compositions of 26 fennel landraces from various growth types and geographical originations were assessed. Essential oil content of the fennel landraces varied from 1.1 to 4.8%, with late and medium maturities showing higher essential oil contents than early maturities. According to the Gas Chromatography Mass Spectrometry (GCMS) results, the main essential oil components were trans-anethole (1.2–88.4%), methyl chavicol (0.2–59.1%), fenchone (1.1–14.7%) and limonene (5.3–15.7%). According to the clustering results, it was noticed that all the fennel landraces originated from arid climates were trans-anethole chemotype with an average concentration of 76%. The early and late maturity fennels from humid climates were methyl chavicol chemotype with an average concentration of 54%. The late maturities from humid and moderate climates were fenchone chemotypes with 12% concentration, and finally the early and late maturities from semi-arid climates were limonene chemotype with 12% concentration. Our results confirm that climate is a major evolutionary determining factor on the phytochemical diversity of fennel landraces.

Key words: Fennel, Essential oil, GCMS, Chemotype.

INTRODUCTION

The oldest evidence referring to usage of herbal medicines and spices traces back to sixty thousands years ago in Shanidar cave in Kurdistan (Lietava, 1992). Fennel (*Foeniculum vulgare* Mill.) belongs to the Apiaceae family. It is an essential-oil-producing aromatic plant and one of the oldest herbs at the global level. Bitter fennel subspecies (*F. Vulgare* Mill. *Vulgare*), possessing appealing flavor and beneficial medicinal effects and is cultivated for source subspecies for the fennel derived drugs (Hornok, 1992). Bitter fennel, hereafter named just as fennel is native to the Mediterranean areas and also has been naturalized in many other regions (Hornok, 1992; Guillen & Manzanos, 1994).

In human nutrition, every part of this plant including seeds, foliage and roots can be used in different ways (Barros et al., 2010; Edoardo et al., 2010). Fennel seed with its

spicy odor and burning sweet taste has a special usage in condiments, perfumes, and industrial liqueurs as a flavoring reagent (Barros et al., 2010; Edoardo et al., 2010; Diao et al., 2014). From the aspect of medical care, the most famous usage of fennel is as an effective anti-colic that's even more effective than chemical drugs (Piccaglia & Marotti, 2001; Alexandrovich et al., 2003). Several studies have shown fennel herbal drugs have valuable antioxidant, anti-cancer, anti-inflammatory, antibacterial, and antifungal properties (Elagayyar et al., 2001; Choi & Hwang, 2004; Lucinewton et al., 2005; Singh et al., 2006; Anwar et al., 2009; El-Awadi & Esmat, 2010; Hamdy Roby et al., 2013; Diao et al., 2014; Ouariachi et al., 2014; Oliveira et al., 2015; Upadhyay, 2015); and several commercial pharmaceuticals are formulated based on fennel essential oil (Elagayyar et al., 2001; Edoardo et al., 2010). In livestock industries, the most significant improvement in a chick's body weight and feed efficiency was obtained by a diet enriched by fennel seed (Mohammed & Abbas, 2009; Teixeira et al., 2013). Naturally essential oil has an important role in attracting insects for pollination (Bowes & Zheljazkov, 2005), however fennel essential oil can be used as a natural pesticide in field and greenhouse crops, and as an anti-mold in foods products (Isman et al., 2011; Regnault Roger et al., 2012; Ebadollahi et al., 2014; Tabrizi et al., 2014). This subject is an important issue in organic food production, and also in cockroach and mosquito control in the human environment (Isman et al., 2011). Some other applications of fennel essential oil are in aromatherapy and massage centers as plant oil (Bowes & Zheljazkov, 2005; Upadhyay, 2015) and in metal industries as a corrosion inhibitor (Lahhit et al., 2011). All the aforementioned pharmacological features and flavoring properties of fennel are due to the presence of certain secondary metabolites in the essential oil; therefore fennel quality is associated with its essential oil content and compositions (Upadhyay, 2015).

More than 90% of fennel essential oil is stored in reproductive organs, especially in seed secretory channels (Akgiil & Bayrak, 1988; Stefanini et al., 2006). According to different studies, the range of fennel seed essential oil content has been reported between 0.6–6% (Bowes & Zheljazkov, 2005; Zahid et al., 2008; Najdoska et al., 2010; Shojaiefar et al., 2015). Essential oil content in the leaves of Iranian fennels had been investigated and ranges from 0.6–2% (Rahimmalek et al., 2014). The main essential oil compositions of fennel are monoterpenes including trans-anethole, methyl chavicol, fenchone and limonene (Singh et al., 2006; Radulovic & Blagojevic, 2010; Shahat et al., 2011). In the study by Rahimmalek et al. (2014), the major essential oil compositions of Iranian fennels leaves were trans-anethole, limonene, and fenchone, with no observation of any methyl chavicol.

Trans-anethole accounts for the anise taste serves as a pleasing aroma in food and perfumes, and as an effective anti-flatulence agent in herbal medicines. Methyl chavicol provides a sweet taste and is mainly used in the perfume industry, while fenchone is responsible for the bitter and spicy taste of fennel, and acts as a real antidepressant. Finally, limonene has well-established chemo-preventive activity against cancers and is also used in resins and solvents (Guilled & Manzanons, 1996; Miraldi, 1999; Pank et al., 2003; Singh et al., 2006; Sun, 2007; Anwar et al., 2009; He & Huang, 2011; Acimovic et al., 2015). According to previous studies about essential oil compositions of bitter fennel, trans-anethole content ranges from 0.1–78%, methyl chavicol 0.1–81.2%, fenchone 1–18.7%, and limonene 1–22%. Trans-anethole has been mentioned as the most dominant component in bitter fennel essential oil (Piccaglia & Marotti, 2001;

Stefanini et al., 2006; Gulfraz et al., 2008; Aprotosoaie et al., 2010; Edoardo et al., 2010; Shahat et al., 2011; Aprotosoaie et al., 2013; Hamdy Roby et al., 2013; Moghtader, 2013; Acimovic et al., 2015; Upadhyay, 2015). Bitter fennel landraces from Asia have higher trans-anethole content rather than from Africa and Europe (Ouariachi et al., 2014), and European fennels have higher methyl chavicol content (Bilia et al., 2002; Edoardo et al., 2010; Basaglia et al., 2014). According to a review by Edoardo et al. (2010), studies about essential oil composition of bitter fennel from Italy have revealed three chemotypes including trans-anethole type, methyl chavicol type, and methyl chavicol/trans-anethole type. According to Ozcan & Chalchat (2006), *F. vulgar Piperitum*, another subspecies of fennel, is mostly methyl chavicol chemotype.

The total world fennel seed production in 2014 was approximately 845 thousand tons (FAO, 2014). In certain industries, the demand for fennel essential oil is rapidly rising; this necessitates the need to develop specific chemotypes. Therefore, fennel needs more attention from researchers, and the first step is to gain the knowledge of present diversity and phytochemical pattern in order to find chemotypes that can guide appropriate selection schemes and breeding programs (Judzentiene & Mockute, 2010).

Among the effective factors on essential oil quality, genetic backgrounds and growth environmental condition are the most important ones (Bowes & Zheljazkov, 2005; Curado et al., 2006; Medina Holguın et al., 2007; Anwar, 2009; Telci et al., 2009; Najdoska et al., 2010; Rahimmalek et al., 2014; Elhassan & Hussein Ayoub, 2014). It is a well-known fact that growth environmental condition can only offer a condition for minor and temporarily changes in essential oil, while the effect of genetic background is major and permanently which is important to us (Bach, 1995; Nemeth, 2005; Medina Holguın et al., 2007; Aprotosoaie et al., 2010). The genetic background of any genome relates to its evolutionary adaptions to the environment in which the ancestors have lived (Amos & Harwood, 1998; Heywood, 2002; Murray et al., 2004; Ramırez Valiente et al., 2009); and that is why botanists describe a healthy gene pool as the one possesses the ability to respond to environmental changes (Amos & Harwood, 1998). Every population experiences a unique environment; hence, a special diversity pattern is expected in each. Fennel populations are widely distributed throughout Iran and have occupied different habitats with diverse ecological conditions. Due to the local environment adaptation, it is assumed each region has its own specific fennel landrace. This subject has been proved morphologically and phenologically (Bahmani et al., 2012a; Bahmani et al., 2015; Shojaiefar et al., 2015), genetically (Bahmani et al., 2012b; Bahmani et al., 2013; Shojaiefar et al., 2015) and cytogenetically (Sheidai et al., 2007).

Identifying the phytochemical pattern in fennel landraces will help to gain accessibility to the desired chemotypes and decrease the threats of genetic erosion. Iranian fennel landraces have never been comprehensively studied for essential oil content and components, so we decided to do this. The aims of this study were: 1. Evaluation of essential oil content and compositions in different Iranian fennel landraces and 2. Identification of phytochemical diversity pattern and specific chemotypes.

MATERIALS AND METHODS

Plant material

In this study, fennel seeds (*Foeniculum vulgare* Mill.) were provided by seed bank of Aburaihan College / Tehran University. This seed bank was founded by the first two

authors of this research in 2010 through gathering ecotypes from different parts of Iran. These ecotypes were separately propagated in the research field of Aburaihan College located in Pakdasht possessing annual averages of 175 mm precipitation and 16.9 °C temperature (Table 1). The same place of growth for the landraces means their differences are only due to genetic dissimilarities. For this study in 2015, we selected 26 fennel landraces from that fennel seed bank, based on two criteria: 1. Landraces should include all growth types (early, medium and late maturity) and 2. Their origins should be different (Table and Fig. 1).

Table 1. Geography profile of originations of the 26 fennels landraces

No	Landraces	Growth type (Day to maturity)	Altitude (m)	Longitude	Latitude	Climate (Koppen climate classification)
1	Sari	Late maturity (240)	23	53 0 E	36 33 N	Semi humid/moderate
2	Kaleibar		1,105	47 1 E	38 52 N	Semi arid/moderate
3	Hajiabad		931	55 55 E	28 19 N	Arid/warm
4	Qazvin		1,278	50 0 E	36 15 N	Semi arid/moderate
5	Chahestan		27	56 22 E	27 13 N	Arid/warm
6	Meshkinshahr	Medium maturity (180)	1,578	47 40 E	38 23 N	Semi arid/cold
7	Fozveh		1,650	51 26 E	32 36 N	Arid/moderate
8	Kohin		1,668	49 67 E	36 36 N	Semi arid/cold
9	Kashan		972	51 27 E	33 59 N	Arid/warm
10	Khash		1405	61 12 E	28 13 N	Arid/warm
11	Moqhan		31	47 55 E	39 39 N	arid/moderate
12	Ardabil		1,332	48 17 E	38 15 N	Semi arid/cold
13	Khalkhal		1,769	48 31 E	37 38 N	Semi arid/cold
14	Damavand		2,000	52 15 E	35 43 N	Semi arid/cold
15	Marvdasht		1,502	52 83 E	29 80 N	Arid/warm
16	Oromie	Early maturity (120)	1,332	45 4 E	37 33 N	Semi arid/cold
17	Fasa		1,288	53 41 E	28 58 N	Arid/warm
18	Shiraz		1,488	52 32 E	29 36 N	Arid/warm
19	Sabzevar		987	57 43 E	36 12 N	Arid/cold
20	Mahalat		1,775	50 45 E	33 91 N	Semi arid/cold
21	Incheboron		460	55 57 E	37 53 N	Arid/moderate
22	Sanandaj		1,350	47 0 E	35 20 N	Semi arid/cold
23	Rafsanjan		1,580	55 54 E	30 25 N	Arid/warm
24	Hashtgerd		1,426	50 43 E	35 65 N	Arid/cold
25	Yazd		1,230	54 36 E	31 89 N	Arid/warm
26	Bajestan		1,265	58 17 E	34 51 N	Arid/moderate
	Pakdasht		1,025	51 67 E	35 47 N	Arid / moderate

Table 1 continues

Pakdasht	Spring	Summer	Fall
Average precipitation (mm)	35.6	4.5	61.8
Average temperature (°C)	19.5	30.3	10.6

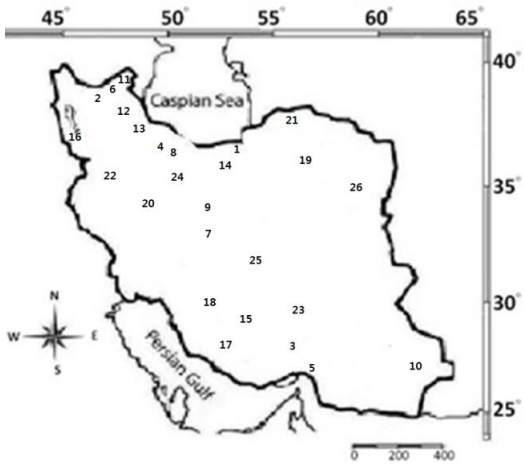

Figure 1. Locations of the 26 Iranian fennel landraces on map (modified from www.enchantedlearning.com).

Essential oil extraction

The essential oil was extracted from the ripened seeds by water distillation in a Clevenger apparatus (Boyadzhieva & Angelov, 2014). After extraction, the essential oils were stored in glass vials in 5 °C until the essential oil compositions analysis by GCMS.

Essential oils composition

In the 26 landraces, determination of essential oils composition were conducted by GC-MS analysis (a combined analytical method to identify different substances within a sample); Varian CP-3800 GC (Gas Chromatography) coupled with Varian 4000 (Ion trap) MS (Mass Spectrometry) equipped with a capillary VF-5 fused silica column (30 m × 0.25 mm i.d., film thickness 0.25 μm). Helium was used as the carrier gas at the constant flow of 1.0 ml min^{-1}; split ratio, 1/50. Mass spectra were taken at 70Ev and Mass range was from m/z 35–400 a.m.u. The oven temperature was held at 60 °C for 1 min, then programmed to 250 °C at a rate of 3 °C min^{-1}, and held for 10 min. The injector and detector (FID) temperatures were kept at 250 and 280 °C, respectively. The essential oil compositions were identified by calculation of their retention indices under temperature-programmed conditions for n-alkanes (C6–C24) and the oil on a VF-5 column under the same chromatographic conditions. The compounds were identified by comparison of their mass spectra with those of the internal reference mass spectra library (Wiley 7) or with authentic compounds and confirmed by comparison of their retention indices with authentic compounds or with those reported in the literature. For quantification purposes, relative area percentages obtained by FID were used without the use of correction factors.

Statistical analysis

In this study, essential oil component yield was calculated by this formula: essential oil content × essential oil component concentration / 100. The graphs were drawn by Excel 2010 and clustering analysis of the landraces was done by SPSS 18.

RESULTS AND DISCUSSION

Essential oil content

The amounts of essential oil content (%) of the landraces were shown in Table 2, and according to that, essential oil content in the studied fennel landraces ranged from 1.1 to 4.8% (cc 100 gr^{-1} seed).

Average essential oil content in early, medium and late maturity fennel landraces were 1.9% ± 0.2, 3.1% ± 0.2, and 3.5% ± 0.4 respectively. Late and medium maturity fennels had the highest amounts of essential oil content. These two were originated from areas with longer and proper growth condition: a circumstance that evolutionary has led them to higher vegetative growth, and consequently higher secondary metabolites production. The same result was obtained from a study by Zahid et al. (2008) saying late maturity fennel landraces with higher vegetative growth have higher essential oil content. The essential oil content in seeds of Iranian fennels is higher than that in their leaves (Rahimmalek et al., 2014). The essential oil contents that we got in this study were almost similar to the previous study on fennel (Bowes & Zheljazkov, 2005; Najdoska et al., 2010; Rahimmalek et al., 2014; Shojaiefar et al., 2015).

Table 2. Essential oil content (%) of the 26 Iranian fennel landraces

Growth type	No	Landraces	Essential oil content (%)	Mean (%)
Late maturity	1	Sari	4.8	3.5 ± 0.46
	2	Kaleibar	4.3	
	3	Haji abad	2.3	
	4	Qazvin	3.5	
	5	Chahestan	2.7	
Medium maturity	6	Meshkinshahr	2.9	3.1 ± 0.25
	7	Fozveh	3	
	8	Kohin	4.1	
	9	Kashan	4	
	10	Khash	3.3	
	11	Moqhan	2.5	
	12	Ardabil	2.2	
	13	Khalkhal	2.4	
	14	Damavand	2.1	
	15	Marvdasht	4.2	
Early maturity	16	Oromie	2.8	2 ± 0.21
	17	Fasa	2	
	18	Shiraz	2.6	
	19	Sabzevar	3.2	
	20	Mahalat	1.4	
	21	Inche boron	2.4	
	22	Sanandaj	2.5	
	23	Rafsanjan	1.1	
	24	Hasht gerd	1.4	
	25	Yazd	1.3	
	26	Bajestan	1.6	

Essential oil compositions

According to Table 3, the results of essential oil GC-MS analysis showed there is a huge qualitative and quantitative difference among Iranian fennel landraces. This diversity has a significant impact on the pharmaceutical and flavoring effects of the essential oil (Aprotosoaie et al., 2013). Based on Table 3, generally, 28 components in our fennel's essential oils were identified. Trans-anethole, methyl chavicol, fenchone, and limonene were the main components existing in all the landraces, constituting 76.7–99.4% of the total values. Additional essential oil components existing in all the landraces were α-Pinene (averagely 1.13%), Sabinene (0.54%), Myrcene (0.79%), α-Phellandrene (0.72%), Ortho-Cymene (0.46%) and γ-Terpinene (0.96%) (Table 3).

According to Table 3, in the Iranian fennel landraces, trans-anethole ranged from 1.2 to 88.4%; methyl chavicol from 0.2 to 59.1%; fenchone from 1.1 to 14.7%; and limonene from 5.3 to 15.7%. This diversity among Iranian fennels is because of genetic dissimilarities (polymorphism) which is in turn due to the diversity of their geographical origin (Bowes & Zheljazkov, 2005; Curado et al., 2006; Medina Holguın et al., 2007; Anwar, 2009; Telci et al., 2009; Najdoska et al., 2010; Kahrizi et al., 2011; Elhassan & Hussein Ayoub, 2014; Rahimmalek et al., 2014). Clustering analysis based on the major essential oil components including trans-anethole, methyl chavicol, fenchone and limonene for the landraces has been done.

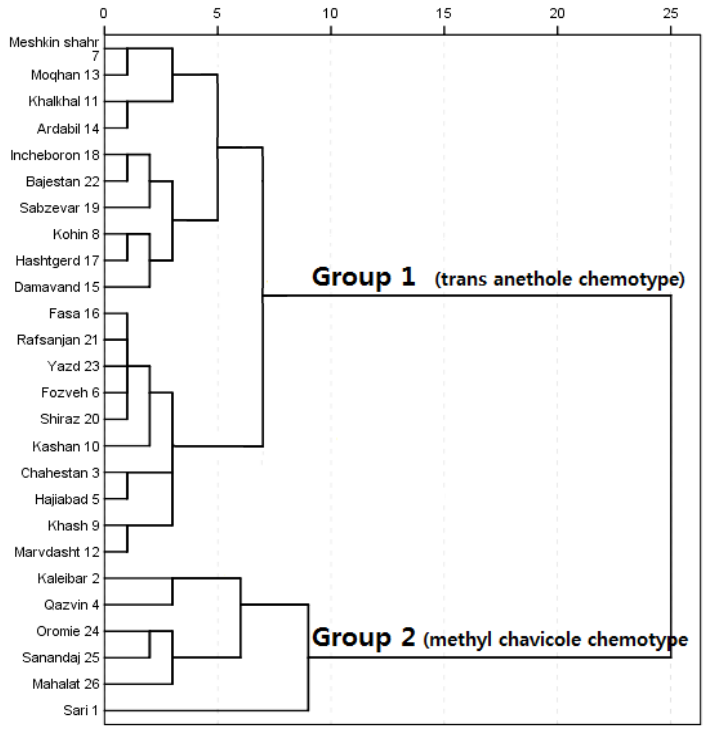

Figure 2. Dendrogram of the 26 fennel landraces with the most important essential oil components using Euclidean coefficient and WARD methods.

Table 3. Essential oil compositions (%) of the 26 Iranian fennel landraces

Composition	RI	Sari	Kaleibar	Chahestan	Qazvin	Hajiabad	Fozveh	Meshkinshahr	Kohin	Khash
α –Thujene	927	0.04	-	0.01	-	0.02	-	-	-	-
α-Pinene	936	1.34	1.02	0.91	1.56	0.6	0.86	0.51	0.21	0.15
Camphene	954	0.2	0.14	0.08	-	-	0.06	0.02	0.08	-
Sabinene	976	0.2	0.12	0.2	1.08	0.71	0.14	0.11	0.14	0.01
β-Pinene	983	0.07	0.04	0.03	0.13	-	0.02	0.02	0.05	-
Myrcene	991	0.97	0.29	0.33	0.88	1.56	0.34	0.17	0.1	0.06
α-Phellandrene	1,011	9.4	0.27	0.08	0.94	0.36	0.08	0.06	0.1	0.01
Ortho -Cymene	1,028	0.83	0.09	0.19	0.9	0.75	0.11	0.15	0.12	0.01
β-Ocimene	1,032	-	-	-	1.2	0.7	-	-	-	-
Limonene	**1,034**	**5.62**	**13**	**5.8**	**11.01**	**5.3**	**9.91**	**8.21**	**9.12**	**6.72**
β-Phellandrene	1,037	0.79	0.09	0.44	0.42	-	-	0.02	0.11	-
Delta.3-Carene	1,037	0.41	-	-	-	-	0.67	0.31	-	0.13
α-Ocimene	1,044	-	-	-	-	-	-	-	-	-
γ-Terpinene	1,062	0.89	1.07	0.87	0.9	0.9	0.89	0.37	0.02	0.09
Fenchone	**1,096**	**14.74**	**9.15**	**6.4**	**9.03**	**5.98**	**3.11**	**3.9**	**4.38**	**4.06**
allo-ocimene	1,128	-	-	-	0.19	-	-	-	-	-
β-Terpinolene	1,144	0.12	0.1	0.04	-	-	0.04	0.03	0.06	-
Camphor	1,154	0.27	0.17	0.07	0.8	0.6	0.11	-	0.016	-
Methyl Chavicol	**1,205**	**55.09**	**59.1**	**1.39**	**52.55**	**1.6**	**3.17**	**17.12**	**10.14**	**0.22**
Fenchyl acetate (endo)	1,222	0.78	0.1	0.11	-	-	0.03	-	0.07	-
Fenchyl Acetate (exo)	1,236	6.17	0.4	0.4	0.45	0.26	0.09	0.02	-	0.03
Cis-Anethole	1,253	-	-	0.03	-	-	-	-	-	0.01
Iso-bornyl acetate	1,287	0.13	-	-	-	-	-	-	-	-
Trans-Anethole	**1,299**	**1.24**	**14.7**	**82.3**	**16.73**	**80.5**	**79.9**	**68.71**	**75.04**	**88.45**
alpha.-Cubebene	1,345	-	-	-	1.3	-	-	-	-	-
Carvotanacetone	1,347	-	-	-	-	-	-	-	-	-
α-Copaene	1,381	0.04	-	0.03	-	-	0.01	-	0.03	-
Germacrene-D	1,487	0.44	0.08	0.27	-	-	0.15	0.16	0.17	-

Table 3 continues

Composition	RI	Kashan	Khalkhal	Marvdasht	Moqhan	Ardabil	Damavand	Fasa	Hashtgerd	Yazd
α –Thujene	927	-	-	-	-	-	-	-	-	-
α-Pinene	936	1.29	3.03	1.03	1.47	1.45	2.01	1.65	2.64	0.2
Camphene	954	0.02	0.28	0.21	-	0.27	0.5	0.2	0.39	-
Sabinene	976	0.12	0.69	0.78	0.59	0.46	0.5	0.44	0.6	1.02
β-Pinene	983	0.04	0.23	0.16	-	0.16	0.24	-	0.16	-
Myrcene	991	0.3	1.44	0.4	0.9	1.34	0.45	1.12	2.03	0.26
α-Phellandrene	1,011	0.11	0.33	0.37	0.66	0.34	0.65	0.31	0.5	0.95
Ortho -Cymene	1,028	0.12	0.87	0.93	1.06	0.7	0.39	0.22	0.35	0.13
β-Ocimene	1,032	-	1.05	1.02	1.33	1.4	1.6	1.8	3.77	0.34
Limonene	**1,034**	**12.4**	**6.54**	**6.8**	**8.1**	**6.62**	**8.5**	**7.41**	**8.14**	**7.22**
β-Phellandrene	1,037	-	1.17	0.62	-	-	0.99	0.57	0.44	-
Delta.3-Carene	1,037	0.81	0.32	-	-	0.21	-	-	-	-
α-Ocimene	1,044	-	-	-	-	-	0.24	-	0.16	0.4
γ-Terpinene	1,062	1.11	1.83	1.51	0.91	1.31	1.84	1.1	1.48	0.98
Fenchone	**1,096**	**2.49**	**4.09**	**4.02**	**3.31**	**4.13**	**4.64**	**1.16**	**4.14**	**2.28**
allo-ocimene	1,128	-	-	0.17	0.24	-	0.31	-	0.22	0.35
β-Terpinolene	1,144	-	-	-	-	-	-	-	-	-
Camphor	1,154	-	0.6	0.76	0.53	0.91	1.13	0.78	0.97	1.58
Methyl Chavicol	**1,205**	**2.5**	**14.35**	**0.45**	**17.28**	**16.15**	**9.59**	**3.4**	**9.97**	**4.1**
Fenchyl acetate (endo)	1,222	0.19	-	-	-	-	0.62	-	-	-
Fenchyl Acetate (exo)	1,236	0.3	1.56	0.45	0.81	0.56	0.9	1.02	-	0.32
Cis-Anethole	1,253	0.05	-	-	-	0.5	-	-	-	-
Iso-bornyl acetate	1,287	-	-	-	-	-	-	-	-	-
Trans-Anethole	**1,299**	**77.63**	**60.39**	**86.17**	**68.74**	**62.28**	**70.26**	**78.43**	**74.28**	**79.25**
alpha.-Cubebene	1,345	-	1.29	0.74	-	1.1	1.65	-	1.43	0.28
Carvotanacetone	1,347	-	-	-	-	-	-	-	-	-
α-Copaene	1,381	0.04	-	0.31	-	-	-	0.37	-	0.08
Germacrene-D	1,487	0.43	-	-	-	-	-	-	-	-

Table 3 continues

Composition	RI	Sabzevar	Shiraz	Rafsanjan	Bajestan	Oromie	Sanandaj	Mahalat	Incheboron
α –Thujene	927	-	-	-	-	-	-	-	-
α-Pinene	936	0.68	0.66	0.9	0.8	1.79	1.04	0.5	1.08
Camphene	954	0.15	0.02	-	0.98	0.43	-	0.024	-
Sabinene	976	0.34	0.43	0.45	2.36	1.26	0.23	0.283	0.76
β-Pinene	983	0.02	0.02	-	-	0.28	0.03	0.053	0.54
Myrcene	991	1.27	0.52	1.23	1.56	1.62	0.28	0.25	0.87
α-Phellandrene	1,011	0.08	0.18	0.66	0.7	0.8	0.12	0.11	0.62
Ortho -Cymene	1,028	0.03	0.02	1.65	0.32	0.37	0.04	0.15	1.52
β-Ocimene	1,032	-	-	1.85	0.86	0.84	-	-	1.48
Limonene	**1,034**	**5.5**	**10.59**	**8.52**	**7.32**	**12.1**	**15.71**	**11.52**	**6.3**
β-Phellandrene	1,037	-	0.65	-	1.01	1.23	-	0.15	0.94
Delta.3-Carene	1,037	0.97	0.51	-	-	-	-	0.48	-
α-Ocimene	1,044	-	-	0.23	0.3	-	-	-	-
γ-Terpinene	1,062	0.08	0.98	0.89	1.9	1.4	0.18	0.47	1.2
Fenchone	**1,096**	**2**	**1.39**	**1.54**	**1.28**	**1.3**	**1.22**	**2.09**	**2.85**
allo-ocimene	1,128	-	-	-	0.53	0.31	-	-	-
β-Terpinolene	1,144	0.02	0.11	-	-	-	-	-	-
Camphor	1,154	0.05	0.15	0.86	2.03	1.14	-	-	-
Methyl Chavicol	**1,205**	**3.4**	**3.5**	**2.49**	**3.12**	**52.27**	**54.98**	**50.89**	**5.71**
Fenchyl acetate (endo)	1,222	-	-	-	-	-	-	-	-
Fenchyl Acetate (exo)	1,236	0.04	0.26	0.26	1.64	1.18	0.03	0.048	0.5
Cis-Anethole	1,253	0.04	-	-	-	-	-	-	0.25
Iso-bornyl acetate	1,287	-	-	-	-	-	-	-	-
Trans-Anethole	**1,299**	**68.96**	**78.98**	**78.16**	**72.66**	**24.79**	**25.86**	**32.69**	**71.65**
alpha.-Cubebene	1,345	-	-	-	0.57	1.9	-	-	-
Carvotanacetone	1,347	-	-	-	-	-	-	-	3.41
α-Copaene	1,381	16.1	0.03	0.28	0.1	-	-	-	-
Germacrene-D	1,487	0.26	0.64	-	-	-	0.23	0.08	-

RI-retention indices relative to C6–C25 n-alkanes on the DB-5 column; t – trace < 0.1%.

As shown in Fig. 2, the 26 Iranian fennel landraces were divided into two groups: Group 1 as the trans-anethole chemotype (averagely 76%) and Group 2 as the methyl chavicol chemotype (averagely 54%). With a closer glance at Fig. 2, we noticed that group 1 includes all the fennel landraces originated from the arid areas of Iran (annual mean precipitation 225 mm) and group 2 includes all the fennel landraces originated from the humid areas of Iran (annual mean precipitation 585 mm). Also, group 1 has two sub-groups; the first sub-group (1-I) includes all landraces from the arid/cold area of southern Alborz (averagely 69%), and the second sub-group (1-II) includes all landraces from arid/warm areas of eastern Zagros (averagely 82%). Group 2 has three sub-groups; the first sub-group (2-I) includes late maturity landrace of Sari from northern Alborz which has a semi-humid/moderate climate in Iran. The second sub-group (2-II) includes early maturity landraces of Oromie, Sanandaj, and Mahalat from western Zagros which has a semi-arid/cold climate. The third sub-group (2-III) includes late maturity landraces of Kaleibar and Qazvin from the middle part of Alborz which has a semi-arid/moderate climate. The three sub-groups of group 2 have almost the same rate of methyl chavicol and the differences among them are related to fenchone and limonene content. Due to the fact that a big part of Iran has low precipitation, most of the Iranian fennels are trans-anethole chemotype. Iranian fennel landraces have been completely acclimatized to the area that they came from. According to Edoardo et al. (2010), Italian bitter fennels were divided into three chemotypes including trans-anethole type, methyl chavicol type, and methyl chavicol/trans-anethole type. In Fig. 3, the topographical features of Iran were shown.

According to Fig. 3, there are two big mountain chains in Iran: Alborz and Zagros Mountain Chains. The climatic features of Iran are the results of these two. Alborz Mountain Chain is like a wall that separates the northern side from the southern side of Iran from the northwest to the northeast of the country and blocks humid winds blowing inland from the Caspian Sea. It provides Iran with a humid northern side, with average annual precipitation of 1,000 mm, and an arid southern side, with average annual precipitation of 250 mm. Zagros Mountain Chain also acts like a wall, running from the northwest to the south of the country, and blocks humid winds blowing from the Mediterranean Sea. It also separates the western side from the eastern side of Iran, and provides Iran with a humid western part, averaging 600 mm precipitation annually, and an arid eastern part that averages 200 mm precipitation annually (Masodian, 2002). In Table 4, details of the 2 main groups and also climatical information of their origins were shown.

Figure 3. Zagros and Alborz mountain chains
(modified from www.worldatlas.com).

Table 4. The pattern of the content of essential oil composition of early, medium and late maturity fennels (climatic data from www.irimo.ir)

Group	Sub-group	Trans anethole (%)	Methyl chavicole (%)	Fenchone (%)	Limonene (%)
1	1-I	**69.29 ± 1.5**	10.68 ± 1.71	3.47 ± 0.34	7.43 ± 0.36
	1-II	**82.47 ± 0.14**	1.67 ± 0.41	4.07 ± 0.58	7.21 ± 0.71
2	2-I	1.24	**55.09**	**14.74**	5.62
	2-II	27.78 ± 2.47	**52.71 ± 1.2**	1.53 ± 0.27	**13.11 ± 1.31**
	2-III	15.71 ± 1.01	**55.82 ± 3.27**	**9.09 ± 0.06**	**12 ± 0.99**

Table 4 continues

Group	Sub-group	Origins	Climate	Annual Precipitation (mm) / air humidity (%) / temperature (°C)
1	1-I	Southern Alborz	Arid/cold	250 / 50 / 10
	1-II	Eastern Zagros	Arid/warm	200 / 45 / 20
2	2-I	Northern Alborz	Semi humid/moderate	900 / 75 / 16
	2-II	Western Zagros	Semi-arid/cold	450 / 60 / 10
	2-III	Middle Alborz	Semi-arid/moderate	400 / 55 / 15

The bold numbers show the highest values of essential oil compositions.

According to Table 4, the highest trans-anethole content was found in the landraces related to sub-group 1-I from eastern Zagros with arid/warm weather (200 mm precipitation and 20 °C temperature) and sub-group 1-II from southern Alborz with arid/cold weather (250 mm precipitation and 10 °C temperature). The evolutionarily determining factors in trans-anethole chemotypes development are firstly dryness and secondly hotness of weather in fennel origin areas. This finding is similar to what Rahimmalek et al. (2014) found out about the positive correlation of trans-anethole concentration in fennel leaves with a high temperature of its origins. We can name the fennels from eastern Zagros as 'the first-grade trans-anethole chemotypes' with 82% trans-anethole content, and those from southern Alborz as 'the second-grade trans-anethole chemotypes' with 69% trans-anethole content.

Apparently all of the medium maturity landraces were trans-anethole chemotype (group 1), while early and late maturities could be trans-anethole or methyl chavicol chemotypes. Rahimmalek et al. (2014) claimed that leaves of late maturity fennels have higher trans-anethole, however, in our study only seeds of those late maturity landraces from arid and not humid climate had high trans-anethole concentration.

Fennel seeds of trans-anethole chemotypes (group 1) are so useful to feed domestic animals. The trans-anethole, which can act as phytosterol, improves the final yield like egg / meat (Piccaglia & Marotti, 2001), so group 1 could be the selected chemotype to produce fennel seed to feed animals.

The highest methyl chavicol content was related to the late and early maturity landraces in group 2 including; northern Alborz (2-I), middle Alborz (2-III) and western Zagros (2-II) which are the most humid areas in the country (averagely 585 mm precipitation). It would be logical if we say humid weather evolutionarily has been the determining factor in methyl chavicol chemotype development. We can name the late maturity fennel landraces from northern and middle Alborz (groups 2-1 and 2-III) as 'the first grade methyl chavicol chemotypes' with 55% methyl chavicol concentration, and those early maturity landraces from western Zagros (group 2-II) as 'the second grade methyl chavicol chemotypes' with 52% methyl chavicol concentration (Table 4).

According to previous studies (Miraldi, 1999; Gross et al., 2009; Raal et al., 2011), there is a negative correlation between trans-anethole and methyl chavicol (which is the same result from our study). Gross et al. (2009) proposed that trans-anethole and methyl chavicol probably have a common biosynthetic precursor. They also reported the action of a bi-allelic gene in the biosynthesis of trans-anethole and methyl chavicol, with partial dominance for high methyl chavicol content. According to Molino (2000), methyl chavicol is produced exclusively through transformation (isomerization) of trans-anethole, but the reverse situation is also possible.

The highest fenchone content was found in the late maturity landrace from northern and middle Alborz (2-I and 2-III). It seems that late maturity and being originated from humid and moderate climate is a good marker for finding fenchone chemotype. We can name those fennels from northern Alborz as 'the first-grade fenchone chemotypes' with 15% fenchone concentration, and those from middle Alborz as 'the second-grade fenchone chemotypes' with 9% fenchone concentration (Table 4).

According to Miraldi (1999) fenchone chemotypes are originated from areas near to sea; in our study, the identified fenchone chemotypes from northern and middle Alborz are very close to the Caspian Sea which gains a lot of humidity and weather is moderate. We should consider that late fennel landraces from the south of Iran are also near to sea but have not been included in fenchone chemotype; the climate in the south of Iran is extremely arid and hot.

A high quality fennel essential oil has fewer than 10% methyl chavicol and fenchone which makes it taste sweet and proper for food industries (De Vincenzi et al., 2000; Bilia et al., 2002; Bowes & Zheljazkov, 2005; Zahid et al., 2008), based on that those landraces from arid areas of eastern Zagros and Southern Alborz (Group 1 in Table 4) are the best ones.

The highest limonene contents were related to landraces from western Zagros (2-II) and middle Alborz (2-III) averagely with 425 mm precipitation and 12.5 °C temperature. Apparently being originated from an area with normal annual precipitation and temperature is in favor of development of fennel limonene chemotype. We can logically name early maturity fennel landraces from western Zagros as 'the first-grade limonene chemotypes' with 13% limonene concentration, and those late maturity landraces from middle Alborz as 'the second-grade limonene chemotypes' with 12% limonene concentration (Table 4).

Positive correlation of methyl chavicol with limonene reported by Raal et al. (2011), was also confirmed that by our study (about group 2-II and 2-III in Table 4).

According to Oliveira et al. (2015) and Singh et al. (2006) those fennel landraces with high concentration of trans-anethole and/or limonene (group 1 and group 2-II) have a high potential of cytotoxic activity against fungi and also tumor cell lines, based on this Iranian fennels should be considered a promising source to develop specific antitumor source.

In fennel species, the content and compositions of essential oil in the same landrace in different years and even seasons are not majorly different (Bernath et al., 1996; Bowes & Zheljazkov, 2005; Stefanini et al., 2006; Aprotosoaie et al., 2010). In this study we assessed 26 fennel landraces from all over the country from all kinds of growth types and originations; so we can universalize these results for other similar geographies and also for fennel plants with different ages.

According to Tables 2 and 3, the landraces with the highest trans-anethole yield were medium maturity landraces (Marvdasht, Kashan, Khash and Fozveh 3 ± 0.2 cc 100 gr^{-1} seed) from eastern Zagros (group 1-II). Landraces with the highest methyl chavicol and fenchone yields were late maturity landraces (Sari, Kaleibar and Qazvin 2.3 ± 0.2 and 0.47 ± 0.11 cc 100 gr^{-1} seed respectively) from northern and middle Alborz (groups 2-I and 2-III). Landraces with the highest limonene yield (0.42 ± 0.3 cc 100 g^{-1} seed) were Kaleibar, Kashan, Sanandaj, Qazvin, Kohin and Oromie.

CONCLUSION

There is a good chance for Iranian fennels to be known and grown as a suitable source of fennel essential oil. Among Iranian fennels, all landraces originated form arid climates were trans-anethole chemotype with average concentration of 76%, those early and late maturity fennels from humid climates were methyl chavicol chemotype with average concentration of 54%, those late maturities from humid and moderate climate were fenchone chemotypes with 12% concentration, and those early and late maturities from semi-arid climates were limonene chemotype with 12% concentration. After finding the desired fennel chemotypes, it is necessary to optimize the environment that we should grow them because we must keep in mind that environmental condition has an effect on essential oil content and composition (Medina Holguın et al., 2007; Aprotosoaie et al., 2010). Our results confirmed that climate is a major evolutionary determining factor on the phytochemical diversity of fennel landraces. According to Piccaglia and Marotti (2001) in fennel landraces the origin latitude which majorly determines the climate has a significant effect on fennel essential oil; this subject also was proved in other plants (Nemeth, 2005). According to Rahimmalek et al. (2009), those yarrow genotypes related to the same chemotypes have been originated from similar climates. There is no direct explanation for these relationships, anyway, changes in regulating genes and enzymes under effects of water and temperature are probably the reason for that (Bach, 1995; Nemeth, 2005). The topic of fennel essential oil is getting more important especially when new usages of fennel essential oil are being found out. Regarding that it is worth to say that fennel essential oil could be extracted even from its callus (Bahreini et al., 2015). This subject opens a new door to those industries that use fennel essential oil and also a new method of enhanced solvent-free microwave extraction can offer numerous advantageous like short extraction time, less energy consumption and lower cost (Benmoussa et al., 2016).

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The dependence of the durability of digestate briquettes and sorption properties on represented particle sizes

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Abstract. Digestate, a product of the anaerobic digestion process, is traditionally used as liquid fertiliser. Besides agriculture use, it became possible to dry its separated solid part and compress it into briquette or pellet form. In the context of the characterisation of briquettes, the description here largely covers the mechanical properties of texture components and the distribution of particles within the briquette space. In order to define these properties and understand the relations between the mechanical part and any influencing factors, researchers started to identify the relationship between particles size distribution in briquettes and sorption properties and therefore mechanical properties. The objective of the present research was to compare size distribution in particles in different digestate samples and to study the connection to water sorption by briquettes and the durability of briquettes that have been made from two kinds of digestate material. For a comparison, two types of digestate were used, for which particles were split into a few size files according to the sieve size. By using digital image analysis, the dimensions of particles were specified and compared with values that were measured by means of a calliper. Sorption properties were defined through experimentation: exposing briquettes to a water source with water adsorption being determined via moisture content. Other mechanical properties were represented by toughness and the rate of abrasion. As result, digestate is an appropriate sorption matter which can multiply its initial mass by a factor of five if the water supply is sufficient. In the case of a dimension measurement of particles, digestate texture is represented by particles with one prevalent dimension, in most cases this being length. The length of particles was between approximately 1mm to 9 mm. The digestate has been proven to be a good water sorbent material and can be applied in various sectors of agriculture.

Key words: anaerobic digestion, absorption, physical properties, distribution, image analysis.

INTRODUCTION

In an attempt to use agro-biological material more productively, a good many disposal technologies are being invented or improved. One of them is the process of anaerobic digestion (AD), which is something that is purposely ongoing in biogas plants (BGPs). The number of BGPs in the Czech Republic is approximately 554 (Amon et al., 2007; EBTP, 2013). This number of plants is quite high and they consume a large volume of biomass matter. The BGPs soon became part of almost every agricultural farm. As part of their operations, a substrate from lower quality grasses and manure was used up, but its quantity was not high enough for a complete view of biogas use to be formulated.

More biomass was needed for full and total operation. This is why, in recent times, maize or grass-cuttings have made up approximately one half of the total mass input, and this plant material is purposely grown for BGP operations (Amon et al., 2007). All BGPs are optimised in order to be able to manage advanced biogas production and therefore provide higher levels of electricity production (Ward et al., 2008). Biogas is then front-product burned in order to provide power to generators that are producing an electric charge, with the rest being a by-product after the process of AD. Digestate is still used in the same way, like a fertiliser (Alexander, 2002) or is separated into 'liquor' and 'fibre'.

'Liquor' usually contains 10% dry matter and, in most cases, is partially used again in the AD process with a new dose of input substrate or is used as liquid fertiliser (Hills & Roberts, 1981; Li et al., 2011; ADBA, 2012). The composition of dry matter in the solid part varies (it is generally between 40–85%) according to input feedstock material. The feedstock material is usually animal manure, slurry, food wastes, energy crops residues, and bio-wastes, so the representation of lignocellulosic components is rich in digestate (Černá, 2015). The solid part of the digestate is commonly used in stables as bedding for animals or is used as fertiliser with additional additives (Alghren et al., 2010). Another use for separated digestate can be in its compressed form (as a briquette or pellet) for direct burning, but this is not such a frequent utilisation because it tends to be primarily long fibre material, so the most useful application is as a soil conditioner (ADBA, 2012). Using digestate for energy purposes is not such a convenient method. The first reason for this is that the process of producing digestate briquettes is more expensive and energy demanding than energy production itself, which is between 15–16 MJ kg^{-1}; it is smaller in comparison with other easily accessible biomass materials that have higher calorific values. Another reason is the higher content of nutrients in matter and ashes, which are more useful as fertiliser than as an energy source (Kratzeisen et al., 2010). Yet another reason to use digestate as fertiliser in a compressed form is the option it provides to mix amendment additives into briquette without any change of the briquette's compatibility, as well as its good sorption properties (Černá, 2015).

Such biodegradable and agro-waste material has a high bulk density, making it hard to store, and therefore it is desirable to compress the biomass to much higher densities, to improve transportation efficiency, better biomass handling, better storage, and the better utilisation of the original matter, which is due to its high moisture content, irregular shape, and sizes that are less appropriate or which are entirely inappropriate for direct use (Zhang & Guo, 2014; Miao et al., 2015).

In an attempt to use such biodegradable agro-waste material in better and alternative ways, excluding for fuel purposes, this paper is focused on the mechanical properties that are represented by inner textural characteristics, specifically by particle dimensions, mainly by size; shape is not closely observed in this paper. Abundantly available agro-waste materials are primary composed of cellulose, hemicellulose, and lignin (Hassanein & Koumanova, 2010). Therefore, the particle shape of such biomass materials is very irregular. Researchers investigated the possibility of particle shape increasing the strength of the material, making it stronger and harder and, putting aside durability or density, it also plays an important role in briquette characteristics (Kakitis et al., 2011). Particles can be distinguished by a spherical (as described in one dimension) or non-spherical shape (using two different dimensions), as illustrated in Fig. 1. Even small changes in the content of fine particles are providing measurable changes in

cohesiveness (Guo et al., 2012). In the study by Hann & Stražišar (2007) it was noticed that the yield strength of bulk solids increased with the size range of particles. Each particle change, along with other changes (mechanical or chemical), such as fibre content, particle size, moisture content, temperature, feed rate, size and shape of die etc, influences briquette properties (Chen et al., 2015). For example, mechanical compression can influence particle structure, including porosity, surface area, and texture, which are important parameters (Tabil et al., 2011a; 2011b). All image analysis projections are in 2D in this paper, with the third dimension not being visible as seen in Fig. 1.

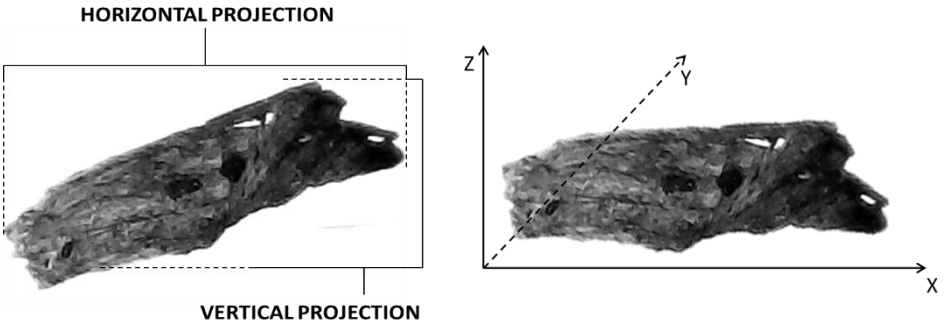


Figure 1. Non-spherical particle in 2D projection.

Lignocelluloses are very effective adsorbents (bonding water to its surface area at the microscopic scale), due to permeated macropores in the matter, which are used for various purposes such as, for example, pollutant absorbers. Porosity can be changed through the process of biomass treating (Miao et al., 2015), and sorption is affected by some parameters such as sorbate concentration, sorbent dosage, and agitation (Hassaneim & Koumanova, 2010). Sorption and moisture content are discussed in this paper because high moisture levels affect briquette cohesion and can result in unwanted swelling and the disintegration of briquette (Singh, 2004).

Particles can be measured by using different approaches, but the image analysis method is the universal method, one that allows texture elements to be compared and quantified and information on materials to be expressed by using quantitative data, such as particle size distribution in graphic or numeric form, or the mean value of monitored variables (Černá et al., 2015). Particle size analysis supplies a variety of approaches for the reporting results. The best way of expressing the result is to describe the width of distribution. As has already been reported, particles influence a number of properties in particulate materials and are an important indicator of quality and performance (Rawle, 2003; HORIBA Instruments, 2014).

The objective of this research is to compare the size distribution of particles in various digestate samples and to analyse the influence on water sorption by briquettes, and the strength of briquettes that are made from two forms of digestate material. The secondary goal of this paper is to observe the relation between the length or width of each particle and its mass.

MATERIALS AND METHODS

The research that has been conducted on digestate briquettes provided the initial characteristics for those briquettes. Those initial characteristics have been noted in Table 1 in terms of diameter, height, volume, and so on. From these values, properties were observed that corresponded to the points below:

- physical and chemical characteristics;
- sorption levels;
- particle measurements.

Research Material

The material being studied – digestate – was obtained from two different biogas plants. Feedstock material for anaerobic digestion and therefore the composition of digestate matter was 40% maize (green), 20% grasses, with the rest being silage from maize, clover, and alfalfa. These samples (D1 and D2) were dehydrated and separated into their solid and liquid parts. Both digestate samples were, after the mechanical dehydration process, additionally dried in the laboratory to the moisture content levels that were required for pressing purposes (to a maximum of 14.5%). The greater part of dried digestate with a moisture content of 13% and 18% was compressed to briquette form and the non-compressed remainder of the digestate (with a moisture content of 9.5%) was used for sieve analysis in order to obtain the size distribution of particles.

The nutrient composition of solid matter was also investigated, and the results are presented in Table 2. All measurements were conducted under laboratory conditions, where the temperature was 20–22 °C and ambient air humidity was in the range of 45–60%.

The properties of briquettes

Before pressing, the structure of the particle weight distribution for digestate (D1) was gained through sieve analysis at an AS 200 screening machine for each particle size fraction corresponding to the sieve mesh's sizes. In Table 3 the mean values are noted from five repeating sieving procedures; one procedure took ten minutes without interruption.

Briquettes were produced using a hydraulic piston press with a working pressure of 12 MPa and the diameter of briquettes was approximately 60 mm (corresponding to the diameter of the pressing chamber). As comparison material for sorption properties, measurements were used that were taken from woodchip briquettes (WCH) made from spruce (*Picea abies* L.) wood chips, and compressed under the same conditions as digestate D1 and D2. In Table 1 the mean values are noted from six repeating measurements.

Table 1. Initial characteristics of briquettes

Briquettes	Diameter, cm	Height, cm	Volume, cm^3	Moisture, %	Density, g cm^{-3}
D1	6.67	4.83	168.55	4.92	0.7869
D2	6.65	4.08	141.79	5.16	0.7689
WCH	6.52	4.11	137.22	5.61	0.7921

The mechanical durability of briquettes was measured by using the abrasion rate according to EN 15210-2:2011 in a standard test drum. An abrasion test for D1 and D2 digestate briquettes was carried out for briquettes with a moisture content of 5.1–5.5%. The percentage of abrasion expresses the cohesion of briquettes in relation to the number of manipulations involving them (the number of drum revs in a given cycle corresponds to the number of manipulations). Briquettes were tested consecutively, five times. Their initial weight was D1 – at 2,043.6 g – and D2 – at 2,061.0 g. In each sample file there were seventeen briquettes of different weights.

The hardness of the briquettes was simultaneously measured with a durometer by using the 'Shore' scale, for the purposes of additional information.

Water sorption measurements

Produced briquettes were left in laboratory conditions for two weeks in order to balance out any difference in moisture levels. Initial values are noted in Table 1. Water sorption was measured simultaneously and repeatedly for D1, D2, and WCH briquettes in special wooden boxes (flow boxes – see Fig. 2). Briquettes were incorporated into the same type of soil in order to simulate soil conditions, with the same initial moisture content and no added water within the process of the experiment. Water sorbed by briquettes was observed and measured by noting any changes in the dimensions of the briquettes and through any changes in moisture content in relation to the time in which the briquettes have been left in the soil. The system simulates fully controlled soil conditions. Briquettes were placed in a flow box in three parallel lines. See Fig. 2 for details. In order to prevent evaporation through the box walls, PVC foil was used.

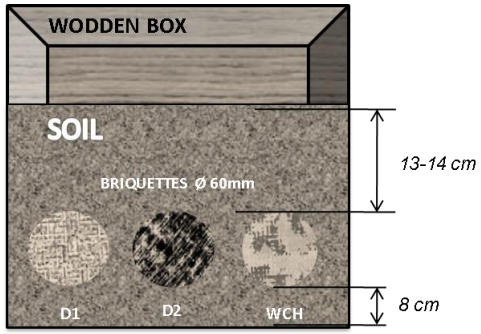


Figure 2. Experiment box for sorption observation.

Particles measurements

Because the process of sieve analysis for both D1 and D2 is very similar, measurements of particle dimensions were conducted only for the D1 sample, for particles passing through a 1mm sieve (particle size were at an interval of 0.5–1 mm). These particles were chosen thanks to the possibility of being able to measure them directly; each particle could be measured in parallel by using image analysis and callipers. Powder particles that make up approximately 1% of the sample are easily measureable by using image analysis, but manually they cannot be measured comparably. Particles were measured physically using callipers and digitally by using image analysis with a Bresser digital USB microscope and ImageJ freeware. The method

used in measuring with callipers is not comparable to that used in image analysis (due to the number of corresponding measured points), but it is an easy-to-handle manual verification of the accuracy of the program settings and of the measuring process itself. This approach was used in order to compare the accuracy both of the measurement methods and the influence on particle density determination; eventually to be able to set out the dependence of briquette strength against the size of the particles.

Length (L), width (B), and thickness (Y) were measured with callipers. Weight (M) was also measured for each particle using scales. Due to the fact that digital images are provided in the planar projection (2D), only the length, width, and area (S) were measured (see Fig. 3). Thickness is invisible for such images. The analysis approach was as follows: firstly, measuring scales were set out and calibrated in the software program, ImageJ; then particles were screened with a microscope camera using appropriate light levels. The screening was sent to ImageJ, where it was cleaned up and converted into a binary image. Particle dimensions such as length and width were measured by hand via the ImageJ program and the image shown of the particles. Once the particle dimensions were measured three times in one direction (point to point) then the values were averaged to a mean value for the given dimension. After obtaining the mean size value of length (L) and width (B) of a particle, the threshold function was applied in order to measure the area (A) of a given particle. The threshold function is defined so that the program could decide when to consider something as being part of the object and when not to do this. Afterwards, the image was passed over to the threshold (this is the simplest method of image segmentation, carried out by transferring the image to greyscale), and the area was measured automatically (Wojnar, 1999). All data points are recorded and can be transferred to an Excel file. Volume (V) and density (ρ) were calculated for each particle from the known values.

The size of sample file D1 contained a total of 64 particles (measured by calliper); while for image testing of the same particles the file was smaller (at 54 particles), due to a difficult manipulation process and the fragility of the particles themselves.

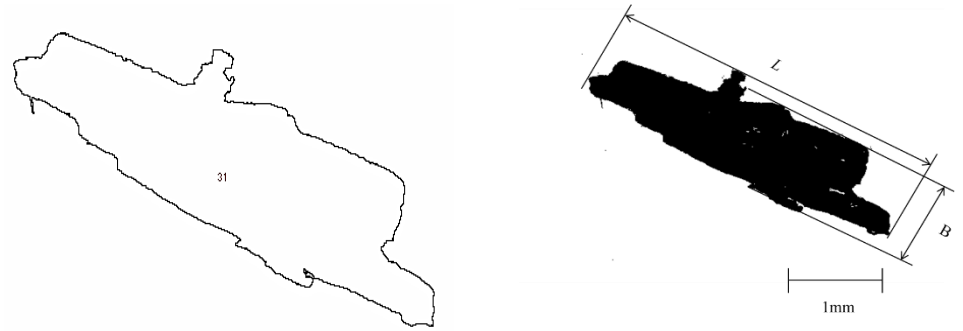


Figure 3. Particle area (S) measurement and particle dimensions – length (L) and width (B) – measurements made with ImageJ software.

RESULTS AND DISCUSSIONS

All of the results have been presented below in tables and/or figures to meet the set objectives. Table 2 shows the values for the content of identified nutrients in samples

D1 and D2 for 100% dry matter. As can be seen here, samples D1 and D2 contain relatively high amounts of fibre (30–35%) and organic matter (88%). From this it can be said that digestate material is fibrous material, and particles are non-spherical in shape (Hann & Stražišar, 2007; Guo et al., 2012). Ash content varies at around 12–13%, which is in comparison to the figure for wood pellets (1.5%), which is ten times higher (EN 14961-1, 2010).

Table 2. Nutrient values of digestate briquettes, 100% DM

[%]	Ash	N·6.25	NFE	Fats	Fibre	OM
D1	11.93	12.27	40.75	0.30	34.76	88.07
D2	12.41	20.30	40.37	1.00	25.92	87.59

*DM – dry matter; N – nitrogen; NFE – nitrogen free extracts; OM – organic matter.

In order to investigate the dependency of both of the textured digestates that were represented by the size distribution of particles D1 and D2 (Table 3 and Fig. 4), and the durability of briquettes as represented by their abrasion levels, it can be noted that any difference in abrasion levels between samples D1 and D2 is probably caused by a greater representation of longer particles in D2, which are notably flat and straight. Such particles do not form a solid mechanical connection after compression. Therefore, they are easier to crumble, which is shown in Fig. 5. In contrast to this, particles from the D1 sample are shorter on average, but they are not straight and their mutual mechanical join in the briquette (their interlocking ability) is therefore stronger (Rawle, 2003; Kakitis et al., 2011; Guo et al., 2012). Moreover, the digestate D2 even has a different value when it comes to adsorbed water in the soil, as shown in Fig. 6. Any difference in the level of abrasion between briquettes D1 and D2 is very obvious in the first round of abrasion tests, because the sharp edges of the briquettes around their perimeter are very easy to break off and, therefore, this process decreases the weight of the sample to a detectable degree. In order to eliminate the time effect on the amount of abrasion in both samples, abrasion was carried out on them in one day. Due to the influence of the environment and various manipulations involved in the use of briquettes, self-abrasion occurs on the briquettes so the suggestion here is to introduce abrasion tests as soon as possible after the creation of digestate briquettes.

Table 3. The structure of distributed particle dimensions in digestate samples D1 and D2

	Size of sieve mesh, mm								
	bowl	0.1	0.25	0.50	1.00	2.50	5.60	10.00	Σ
No.	1	2	3	4	5	6	7	8	
D1, g	0.55	1.02	2.38	3.12	10.96	17.25	10.74	19.14	65.31
%	0.86	1.56	3.65	4.79	16.84	26.42	16.50	29.40	100.0
STD1	0.11	0.19	0.46	0.74	1.00	1.15	1.09	3.60	
D2, g	0.98	1.54	3.44	9.00	22.82	27.59	22.64	14.14	102.15
%	0.96	1.50	3.36	8.81	22.34	27.03	22.16	13.84	100.0
STD2	0.08	0.28	0.57	2.03	3.20	1.67	2.30	8.97	

*STD – standard deviation.

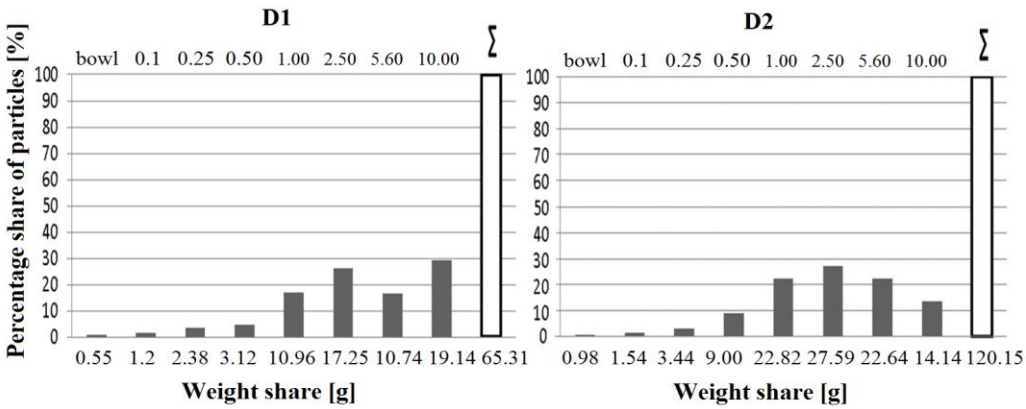


Figure 4. Illustration of weight distribution across the whole sample (D1 and D2) ordered according to the size of sieve mesh in % terms.

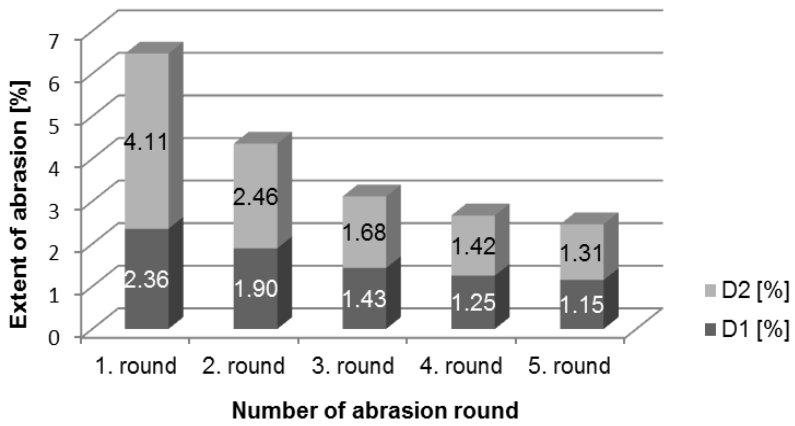


Figure 5. Abrasion testing for D1, D2 expressed in % terms.

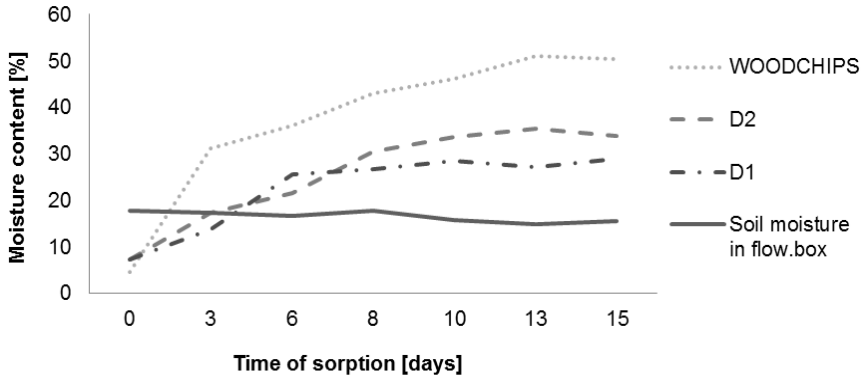


Figure 6. The progression of water sorption by D1, D2, and woodchips briquettes in soil conditions.

The influence of particle size and shape on the durability of briquettes (Miao et al., 2015) is demonstrated in the assumption that abrasion in briquettes D1 and D2 is carried out in the same way and by using the same piece of equipment. Therefore, the abrasion tests that have been carried out represent more 'coherence' in terms of the briquettes that are being used and thereafter also more coherence in their durability. It can be seen that the abrasion rate represents the handling of briquettes rather than anything else. Each round of briquette abrasion represents a number of manipulations, where one round is approximately 105 rotations which, theoretically, is the equivalent of the total number of drum rotations managed by the test equipment. The first and second rounds are the most significant thanks to a large difference in the abrasion extent results, which were caused by higher levels of abrasion on the sharp edges; this tendency has also been observed in another study that was published by Brunerová et al., in 2016. This can be explained as the actual kinetic energy of the briquette sample after it hits the internal drum surface and converts into mechanical energy, which causes abrasion on the briquette sample, which can then be comfortably measured as a loss of its weight. It should be borne in mind that abrasion values are average ones because it is impossible to monitor with any great accuracy the abrasion of individual briquettes in the sample.

Briquettes that have been incorporated into the soil are consistently showing very high values in terms of sorption progress, as proved by the experiments carried out by Pecen et al. (2013), where the sorption properties of different materials are compared. The briquette material is essentially an aspect of the water uptake volume, and the dimensions of pressed particles and the compression force being used are important factors, as described in Tabil et al. (2011a) and Chen et al. (2015). All of these factors, including the process of depositing the briquettes in soil, the technology used in briquette production, and the conditions under which such production was carried out were the same both for digestate and woodchip materials. Therefore there is only one variable – the materials themselves. Fig. 6 shows that both samples of digestate have similar water uptake levels. Briquettes, both from digestates and woodchips, show a similar water sorption start uptake (Pecen et al., 2014; Černá, 2015; Černá & Pecen, 2015), but after about ten days the water sorption by briquettes practically stops; different values for different briquette materials were achieved. This final phase is characterised by an equilibrium of conditions between briquette moisture levels and soil moisture (Singh, 2004). It should be noted that this is mechanical and physical sorption, which is dependent mainly upon the texture of the briquettes. Fig. 6 shows that the visible changes in soil moisture levels (between the beginning and end of the experiment) are very small in comparison to any changes in briquette moisture levels (Pecen et al., 2014). This pattern of sorption is very similar from repeated attempts at the process (Černá, 2015). Therefore, water sorption data are expressed as average values. Fig. 6 well illustrates the differences in water sorption levels between briquettes that are made using different materials. Woodchip briquettes have smaller density levels and then accept a higher water content when compared to digestate briquettes with a higher density level. The progress shown in the graph is the same in both digestates, D1, D2, and WCH samples. The smallest difference was noticed in sorption by soil during the progression period.

Considerable attention was paid to an analysis of digestate particle size. This area was examined in two ways: first using digital callipers (with a resolution of 10 μm), and second electronically, using the image analysis program, ImageJ.

The length and width of each particle was measured electronically. From this data, the area of each particle was calculated. Surface area was also directly measured with this method. Callipers were used to measure the length, width, and height of each particle. From this data, the volume and density of each particle was calculated. The weight of each particle was determined using scales, with a resolution of 0.1 mg. The height data for particles were used in calculating the volume and density using the applied method of image analysis. As a model material for a detailed analysis of particle size, digestate D1 was used. A particle size fraction of 0.5–1 mm was measured from the sieve analysis. The resultant mean values are listed in Tables 4 & 5, including the calculated differences for area, volume, and density (Table 6).

Table 4. D1 particle dimensions as measured using callipers

Calliper measuring							
Parameter	Length	Width	High	Area $L_0 B_0$	Volume	Density	Weight
Abb.	L_0	B_0	Y_0	S_0	V_0	ρ_0	M
Unit	mm	mm	mm	mm^2	mm^3	$g \cdot cm^{-3}$	g
No.	1	2	3	4	5	6	7
$MEAN_A$	3.94	0.98	0.27	3.78	1.11	0.5573	0.0005
MAX	8.25	1.68	0.55	9.49	4.26	5.4681	0.0017
MIN	1.13	0.27	0.04	1.30	0.05	0.1899	0.0001
STD	1.63	0.28	0.13	1.78	0.90	0.6667	0.0003

*****Area $S_0 = L_0 \bullet B_0$| Volume $V_0 = L_0 \bullet B_0 \bullet Y_0$.

Table 5. D1 particle dimensions as measured using image analysis

Image analysis measuring							
Parameter	Length	Width	High	Area $L_1 \cdot B_1$	Volume $S_1 \cdot Y_1$	Density	Weight
Abb.	L_1	B_1	Y_1	S_1	V_1	ρ_1	M
Unit	mm	mm	mm	mm^2	mm^3	$g \cdot cm^{-3}$	g
No.	8	9	10	11	12	13	14
$MEAN_A$	4.03	0.84	0.27	3.37	0.88	0.6977	0.0005
MAX	8.79	1.44	0.55	9.98	4.09	5.7596	0.0017
MIN	1.19	0.27	0.04	0.76	0.05	0.2059	0.0001
STD	0.15	0.26	0.13	1.75	0.78	0.8197	0.0003

***** Area $S_1 = L_1 \bullet B_1$ | Volume $V_1 = S_1 \bullet Y_1$.

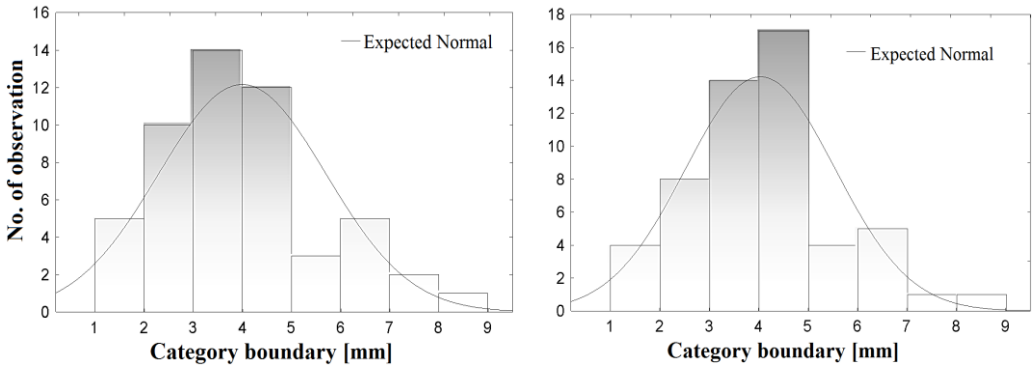
Table 6. Differences between calliper and image analysis measurements

	Difference		
Parameter	Area	Volume	Density
Abb.	ΔS	ΔV	$\Delta \rho$
$MEAN_A$	-0.9215	-0.2151	0.0330
MAX	4.2538	0.9770	2.1222
MIN	-6.1280	-2.4484	-0.7101
STD	1.9365	0.5426	0.3828

***** $\Delta S = S_1 - S_0$ | $\Delta V = V_1 - V_0$ | $\Delta \rho = \rho_1 - \rho_0$

The uncertainty involved in measuring with callipers is in the order of a few percent (0.1 mm); in the case of image analysis the uncertainty value is smaller (0.01 mm).

Fig. 7 shows the frequency distribution (histogram) of the measured particle length values for the D1 sample for both measuring methods (digital callipers L_0 and image analysis L_1).



Summary statistics: L_0
N = 52, Mean = 3.995, Minimum = 1.13,
Maximum = 8.25, Standard deviation = 1.705

Summary statistics: L_1
N = 54, Mean = 4.032, Minimum = 1.19,
Maximum=8.79, Standard deviation = 1.515

Figure 7. Histograms of length L_0 (left) and L_1 (right) values for digestate D1 including statistics and expected normal values.

Fig. 7 demonstrates that both methods of measurement are appropriate in the assumption of normal distribution for the sample (measured values for particle lengths). A similar result can also be obtained for the width and height of individual particles (Rawle, 2003).

The results of particle size measurement using image analysis are more accurate, as confirmed in Fig. 8. This is ultimately reflected in the calculated higher value of particle density, which is closer to the average density value as calculated from the determined weight and volume of briquettes, as noted in Table 1. The average density value as calculated from the briquette weight and volume depends mainly upon the levels of pressure used; briquette density increases alongside increased working pressure.

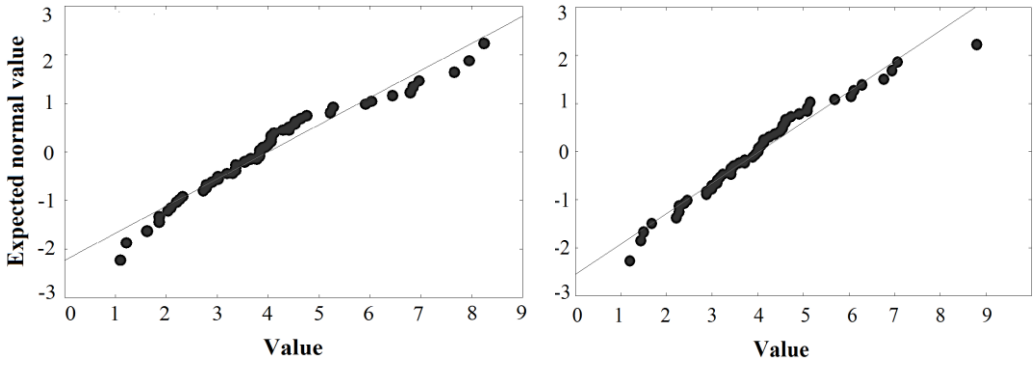


Figure 8. Normal P-plot for length L_0 (left) and length L_1 (right) values for digestate D1 and their expected normal values.

The size and shape of digestate particles also affects the average density of briquettes, toward the higher values (Hann & Stražišar, 2007). The influence of particle size and shape on water sorption by briquettes is illustrated in Fig. 6, where D1 briquettes exhibit less water sorption than D2. Particles in sample D2 are longer and straighter and therefore do not form compact briquettes, whereas in D1 the briquettes have less space between the individual particles. Therefore, in D1 briquettes water sorption is smaller when compared to D2 briquettes. The influence of particle size and shape is also reflected in the abrasion value, as shown in Fig. 5. D1 briquettes therefore exhibit less wear than D2 briquettes.

CONCLUSIONS

The rate of abrasion of digestate briquettes depends primarily on the material properties of the briquettes themselves. The size and shape of particles in uncompressed digestate also have a significant influence on the durability of the briquettes. Particles that are of one dimension longer (plate particles) are, in digestate briquettes, bounded with a smaller force between particles, so that briquette fragments are easily released. Abrasion comparison (resistance in the compatibility and cohesion of compressed particles) between the materials being studied on the basis of fraction distribution was not observed and is recommended as a point to include in the next round of research. Previous research experience shows that the biggest particles in digestate briquettes have higher rates of abrasion and even sorption ability, ie. they are less stable.

Water sorption in briquettes that were incorporated into the soil and the speed of such sorption depends largely on the type of materials used for the briquettes and partly on particle size. Woodchip briquettes were approved as being better water sorbent briquettes when compared to digestate ones, but the difference is no more than a maximum of 10%. While initial soil moisture is not decisive in achieving constant moisture in briquettes, which occurs up to approximately ten days after the briquettes have been deposited in the soil, the moisture levels in the briquettes at this stage is always significantly greater than in the surrounding soil.

An analysis of digestate particle sizes as measured with a digital calliper and using image analysis confirmed the normal distribution of particle size in the sample, and also showed the higher accuracy levels in the determination of particle size when using image analysis. The influence of particle size, especially the length of particles (which, on average, are at a scale of 4.5–5 mm), and shape, was reflected in the measured values of durability and water sorption in those briquettes that were being stored in the soil.

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The technology of soft cheese with a vegetable component

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Abstract. In products with complex composition milk and vegetable raw materials are used in different combinations, which allow to give them certain functional properties. Increasing the production of biologically wholesome products is a highly topical issue. One of the possible solutions of the problem is combining milk basis with vegetable raw material. Studies have been conducted on the development of soft cheeses from goat's milk with chickpea flour. The aim of this research is to study the properties, consumer value and possibility of creation of soft cheese formulation with chickpea flour. In this field of study, an extruded chickpea flour is an innovative additive that had never been used before. Optimal proportion of ingredients was determined by nutritional, biological and energy value under the limitations arising from structural and parametrical models of adequate nutrition. The optimal concentration of bean filler in cheese mass that allows for the insignificant change in qualitative indicators of lacto–vegetarian product (taste, smell, consistency and color) was determined. During the experiments an effective fracture of bean component was selected and qualitative indicators of the developed soft cheese were determined. The paper gives scientific substantiation for the effectiveness of manufacture of soft goat cheese with chickpea flour.

Key words: goat milk, soft cheese, bean filler, chickpea flour, extruded chickpea.

INTRODUCTION

Nutrition is the factor of utmost importance that defines nation's health as a whole and health of the individuals in particular (Asafov, 1999).

The number of research conducted on development of new types of soft cheese due to their technological and economical superiority in comparison to hard and pickled cheese has increased recently (Heinlein & Caccese, 2014). In this regard, development of combined soft acid-rennet cheeses is highly promising. Production of functional products is a topical problem concerning contemporary food industry, particularly dairy industry.

In recent years, both qualitative and quantitative shortage of vegetable components in the structure of nation's nutrition has occurred, thus an increase in biologically wholesome food production has become rather actual (Gavrilova, 2004). One of the possible solutions to the problem is combining milk basis with vegetable raw materials (Gorlov et al., 2012).

Primary and most widespread type of raw material used in dairy product production industry is cow milk (Iakovchenko & Silantjeva, 2014). Cow milk is one of the most important food products of mankind (Vasilyev, 2001; Hennelly et al., 2006). However, it seems reasonable and advisable to use not only cow milk, but also goat milk due to its

hypoallergenic and biological properties. It is well known that goat milk corresponds to physiological features of human organism better than cow milk. Goat milk contains mineral salts in certain proportion and balance. In comparison to cow milk, goat milk contains six times more cobalt, which is a part of B12 vitamin, a lot of calcium, ferrum, manganese and cuprum (Chechetkina et al., 2013). The percentage of goat milk of total milk consumption has significantly increased during recent decades in Russia. Therefore, more indepth knowledge about the composition and properties of goat milk is needed, especially in the context of dairy production (Haenlein, 2004; Sanz Ceballos et al., 2009). According to studies, carried out in different countries, fat content in goat milk ranges from 2.75% (Jandal, 1996) to 5.23% (Sanz Ceballos et al., 2009) and protein content from 2.98% to 3.66% (Strzałkowska et al., 2009). Depending on the stage of lactation and goat breeds, an average content of casein in goat milk varies from 1.06–3.01g $100g^{-1}$, lactose content from 3.85–5.46 g $100g^{-1}$, total solids from 9.8–15.9 g $100g^{-1}$ (Salem et al., 2004; Strzałkowski et al., 2009).

The paper considers the possibility of using chickpea filler as a resource–saving component and functional additive. A high–protein crop such as chickpea is a source of dietary fibers, vitamins, mineral elements, essential fatty acids and phospholipids. Chickpea rivals every other crop containing lysine, tryptophan and aliphatic sulfur–containing α–amino acid–methionine that participates in biosynthesis of adrenaline, choline and cysteine. In order to increase the amount of water-soluble antioxidants the chickpea had been previously sprouted. Sprouted chickpea was extruded. The extrusion allowed enhancing the product's taste due to cleavage of starch into simpler, sweeter components and dissipation of unpleasant smell typical for chickpea. In the process, a new structure appears in the form of biopolymer food foam that eases the product digestibility (Martinchik & Sharikov, 2015).

Based on the foregoing, the aim of this research is to develop the innovative technology of soft cheese production using goat milk and chickpea flour, which is achieved by directed varying of proportions of the raw materials.

MATERIALS AND METHODS

Materials that used for the research are samples of soft cheese products with chickpea flour. The control sample was produced from fresh goat milk that meet the Russian national standard (2008).

Starters: CBL 6−CHEESE, Italy (direct vat set starter with combination of *Lactococcus lactis subsp. lactis/cremoris, Leuconostoc species* (type *L*) or *Lactococcus lactis subsp. diacetilactis* (type *LD*) cultures.

Ferment: calf rennet in the amount of 0.2% of milk volume (1% solution (w/v), obtained from Moscow factory of rennet (TS 10–02–824).

Vegetable component: finely ground extruded chickpea flour, obtained from Volga Research Institute of meat and dairy products of Russian Agricultural Academy, Volgograd.

Total-solids, total protein, fat content were measured using milk analyzer 'Klever−2M' (OOO NPP Biomer). The 'Klever−2M' milk analyzer is designed for measuring the mass contents of fat, protein, lactose and density in milk and dairy products.

Cheese manufacture

Raw whole goat milk was obtained from a local market. The process was carried out according to the conventional technology of soft fresh cheese manufacture. Raw goat milk was pasteurized at 72–75 °C for 20–25 seconds in a water bath, cooled to 28–30 °C and subjected to acid–rennet coagulation. The acid–rennet coagulation of proteins was conducted by using the following components: direct–set bacterial concentrate with a certain combination of *Lactococcus lactis subsp. lactis/cremoris*, *Leuconostoc species* (type *L*) or *Lactococcus lactis subsp. diacetilactis* (type *LD*) cultures in the amount of 1.0% of the mixture; rennet powder (TS 10–02–824) was added at concentration of 0.001 g 100 g^{-1} of milk; calcium chloride (TS 6–09–4711) based on the addition of 40 g of anhydrous salt per 100 kg of the mixture. The mixture was stirred and left for coagulation and acidification at a temperature of 35−40 °C for 90 minutes. After the complete coagulation, the curd was cut into cubes 1 x 1 cm and settled for 10 minutes. Curds were stirred carefully during the next 15 min and settled for 10 min. The chickpea flour was added after the partial whey drainage. Soft cheeses containing different amounts of chickpea flour were produced. The following formulations were produced, in triplicates (three different batches of the same formulation): the amount of chickpea flour varied from 1% to 7% with the increment of 2%. All samples were analyzed in 3 parallel replications. All results were expressed as mean values of three replicate trials. The control sample did not contain any chickpea flour. After that, the whey was drained and curds were transferred into perforated moulds for draining and held for self-pressing at 16–18 °C. The samples of cheese were turned upside-down three times during the first 5 h of draining and self-pressing.

Organoleptic evaluation

Samples were evaluated for organoleptic characteristics by a taste panel of 11 staff members and students of the Applied Biotechnology Department.

The participants were selected and trained in accordance with the ISO 8586-1 standard (1993). Requirements for the work of the group assessors were according to the ISO Standard 8589 (2007). Sensory evaluation of the soft cheese samples was carried out using a quantitative descriptive (profile) of the method for analytical evaluation of dairy products according to the ISO Standard 22935 (2009).

The aim of the organoleptic evaluation of soft cheese was to determine acceptable concentrations of chickpea flour used in soft cheese production. The quality of the cheese curds were evaluated for appearance (colour, colour homogeneity), consistency and texture (hardness, and flavour (odour and taste) using a 5-point scale (Pereira et al., 2011). The participants were asked to assess a number of specific attributes. The cheese curds were randomly coded with three-digit numbers.

Product yield

Cheese yield was evaluated by weighing the samples of soft cheese with and without chickpea flour and presenting the difference in percentage. Experiments were repeated 5 times. Cheese yield from vat milk (CY_v) was calculated using Eq. 1.

$$CY_v = \frac{M_{ch} \cdot 100}{M_m}. \quad (1)$$

where: CY_v – cheese yield from vat milk, g 100 g^{-1} milk; M_{ch} – mass of cheese, kg; M_m – mass of vat milk, kg.

Qualitative properties

To minimize significant inaccuracy, there were 3 experiments for each sample. During the conducted research changes in the main qualitative indicators of cheese products stored at t = 4 ± 2 °C for 10 days were measured.

The titratable acidity was analyzed according to AOAC (1998). Moisture content was determined using standard methods according to the Official Methods of Analysis of AOAC International. These methods of moisture content and total solids determination are based on the sample heating at constant temperature and the loss of weight is used to calculate the moisture content of the sample according to AOAC method 948.12 (2002).

Microbiological indicators such as total viable counts of mesophilic aerobic and facultative anaerobic microorganisms were analyzed according to the AOAC International standard procedure as well. These indicators are the measure of product's quality and safety, its suitability for human consumption.

Microsructure studies of cheese

Freezing microtome (Microm) HM 525 with a freezer temperature at 30 °C below zero was used in the preparation of samples. The samples were strained according to the standard method using hematoxylin and eosin. A microscope AxioCam MRc 5 Imager Z2 was used for the examination of 20 mkm histological slice of the samples that was being held online with the use of the AxioVision computer program. Images of cheese product microstructure with chickpea flour additives were taken.

Statistical analysis of the data

All experiments were performed 3 times. The obtained data were processed by methods of mathematical statistics at theoretical frequency 0.95. The normal distribution and equality of standard deviations of variables were tested with Shapiro–Wilk test and Levene test, respectively. All tests were performed using Statistica 7.1 (StatSoft. Inc., Tulsa, USA) software.

RESULTS AND DISCUSSION

Studies on the regularities of biotechnology formation of cheese product manufacture using vegetable raw materials were conducted. Proportions of the product's composition were selected; the amount of chickpea flour was justified with regard to product being able to maintain necessary organoleptic, physical and chemical, microbiological and rheological indicators.

The main components of goat and cow milk are shown in Table 1. Fat content of the studied goat milk varied from 3.05% to 5.10%. The average fat content was 4.1%

(Table 1), which is somewhat higher than in a study conducted in the US (Park et al., 2007).

Table 1. Ranges in level, average fat and protein content of goat and cow milk

Component, %	Ranges in level, %		Average, %	
	goat	cow	goat	cow
Fat	3.05–5.10	3.80–5.0	4.1	4.4
Protein	2.69–3.95	3.13–3.81	3.32	3.47
Casein	2.61–2.71	2.6–2.7	2.66	2.65
Lactose	4.29–5.0	4.7–4.9	4.64	4.8
Solids	11.14–13.46	12.5–12.6	12.3	12.55
Solids non-fat	8.27–9.14	8.8–9.4	8.7	9.1

Protein content of goat milk ranged between 2.69% and 3.95%. The average protein content was 3.32%. Differences in the results can be attributed to regional peculiarities, breeding conditions, feeding, etc. (Salem et al., 2004; Park et al., 2007; Sanz Ceballos et al., 2009; Rewati Raman Bhattarai, 2012). When comparing the average fat and protein content of goat milk with corresponding parameters of cow milk, it turned out that the average fat and protein content of goat milk was slightly lower.

Organoleptic evaluation of the product

Organoleptic properties are a part of the complex of indicators that determine the nutritional value of cheese products. Table 2 presents samples of cheese produced in triplicates (three different batches of the same formulation): the amount of chickpea flour varied from 1% to 7% with the increment of 2%. All samples were analyzed in 3 parallel replications. All results were expressed as mean values of three replicates.

Table 2. Evaluation of experimental samples of cheese with different mass fraction of chickpea flour

A sample of soft cheese	The amount of chickpea flour, %	Consistency	The average rating
Control	0	gentle, elastic	4.8
Sample 1	1	uniform	3.9
Sample 2	3	moderately dense	4.5
Sample 3	5	gentle, connected	5.0
Sample 4	7	friable	4.4
Sample 5	10	crumbly	3.7

The scoring evaluation of experimental samples of cheese with different mass fraction of chickpea flour showed that all the samples had high ratings; however, the consistency of soft cheese with extruded chickpea in the amount of 5% received the highest score. Thus, taste of extruded chickpea flour in this product is felt softly.

According to the results of the experiments, in the case of using chickpea flour in concentration 5% the product with high organoleptic characteristics was obtained. With the addition of chickpea flour in the range of up to 5%, the consistency, color, taste and smell did not change significantly and were evaluated highly. While the increase of concentration of chickpea flour from 5% to 10% led to the decrease in average scores of mentioned indicators (Fig. 1).

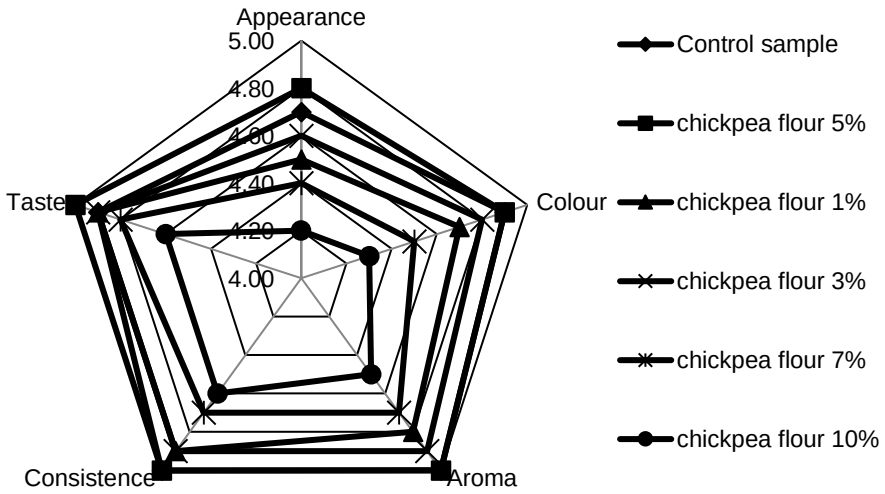

Figure 1. Dependency of point-based cheese product evaluation on mass fracture of the chickpea flour.

The data obtained from organoleptic analysis presented in Table 3. They demonstrate the differences of the experimental sample from control product.

Table 3. Organoleptic indicators of cheese product

Indicator	Control sample	Sample with bean filler
P	Pure diary sour, moderate salty without off-flavors; the surface is moist without mucus	Moderate salty typical for bean filler, weak smell of bean filler; the surface is moist without mucus
Consistency	Soft homogeneous; a little brittle, but not crumble	Homogenous, moderately dense, but not crumble
Pattern	No pattern. Some small round, oval or angular-shaped inclusions	No pattern. Some bean filler inclusions
Color	Uniform, from pure white to light yellow	Pure white, with uniform distribution of bean filler throughout the cheese body

The results of the sensory assessment of cheese quality are given (Fig. 1 & Table 3). It can be seen that appearance, taste and odour, consistency and texture of cheese were affected by the concentrations chickpea flour. The control cheese was a little firmer than cheese with increasing concentration of chickpea flour. The sensory attribute such as colour was found to be significantly different between control sample and samples with chickpea flour addition. The concentration increase up to 5% led to insignificant decrease in cheese firmness. The increase of concentrations of vegetable additive more than 5% led to a gradual decrease in organoleptic characteristics. The consistency of cheese became weaker, porous and even deliquescent. The taste and flavor of chickpea flour were strongly pronounced. All that makes these cheese curds the least acceptable for manufacture of cheese.

Thus, the most acceptable concentrations of chickpea flour for cheese manufacture are 5%. Further concentration increase leads to deterioration in the consistency of the cheese, which complicates soft cheeses obtaining, makes them unacceptable to the consumer.

The coagulation process of the mixture is one of the most important processes in cheese manufacture. Its success depends on a number of factors, as described by many authors (Smirnova & Ostroumova, 2006). The most indicative effect of chickpea flour on the acid accumulation process of samples can be demonstrated for the acid-rennet coagulation method of dairy mixture.

As a result of the research on soft cheese samples, it is shown in Fig. 2 that moisture–binding capacity of cheese with an addition of chickpea flour is higher than that of the control sample. These results demonstrated the efficiency of this additive for regulation of technological properties and quality of soft cheeses. During the cheese storage under constant temperature there was a decrease in moisture content. (Fig. 2).

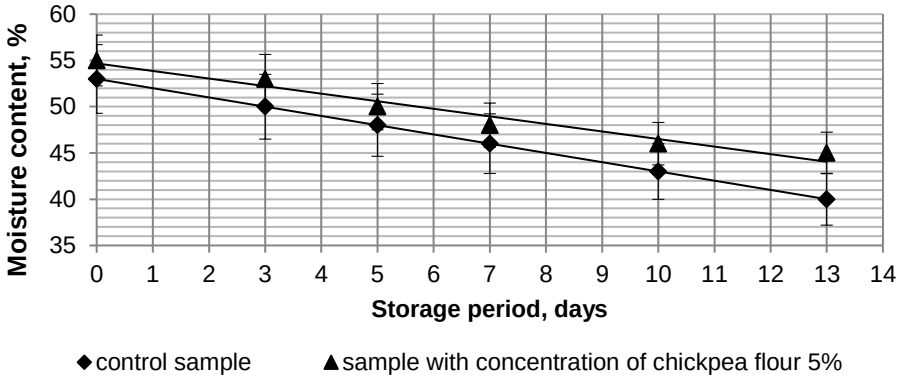

Figure 2. Changes in moisture content of cheese product during storage.

It can be caused by decrease in volume of the aqueous phase by addition of a chickpea flour to milk samples. Increased softness of cheeses made with a chickpea flour can be due to the filler and increased moisture level resulting from the high moisture – binding capacity of the chickpea flour. This can be explained by the positive influence of chickpea flour associated with an increase of hydrophobicity of proteins.

The ability of curds to whey separation during self-pressing process is important in the production of soft cheeses. It is obvious that samples with chickpea flour had a lower ability to separate whey. It was observed that the clots with chickpea flour have separated whey worse than the control sample. The varying water-holding capacities of curds influence the quality of finished products mainly the consistency of cheeses. The samples with chickpea flour were more similar to the control sample according to the ability to separate whey.

Data for titratable acidity changes throughout the storage period are illustrated in the Fig. 3. During the cheese product storage, an increase in acidity value depended on storage temperature and method. Cheese stored in our case at $t = 4 \pm 2$ °C and relative humidity from 80 to 85%.

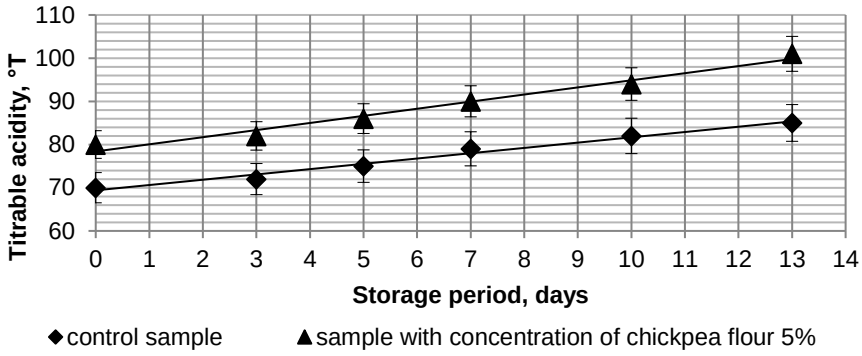

Figure 3. Changes titratable acidity of cheese product during storage.

Fig. 3 shows the increasing trend of titratable acidity of samples with chickpea flour during storage. The increase of acidity during storage was uneven. Acidity changes of the control sample were not significant during the first three days, then after 3 days of storage the acidity started to increase quickly and after 7 days it reached the acidity of the finished product (90°T), and after 13 days – the maximum 101°T. The increase of the titratable acidity of the finished product was more intense at the beginning of the storage period (first 3 days). It was observed that in a sample of cheese with chickpea flour the oxidation process proceeded more intensively. It can be seen from the data presented in Fig. 3, the addition of a chickpea flour slightly increased titratable acidity. Although, it had a tendency of acidity increase with the addition of a chickpea flour. Apparently, it can be due to bacteria, used the filler as a nutrient medium to produce lactic acid. Dynamics of accumulation of lactic acid in the experimental and control samples were similar.

During the product storage there was an increase in the quantity of mesophilic aerobic and facultative anaerobic microorganisms (QMAFAnM). The data presented in Fig. 4 show that the QMAFAnM increase is more intensive in the sample with the chickpea flour. This indicates that the added filler is an additional growth medium for microorganisms.

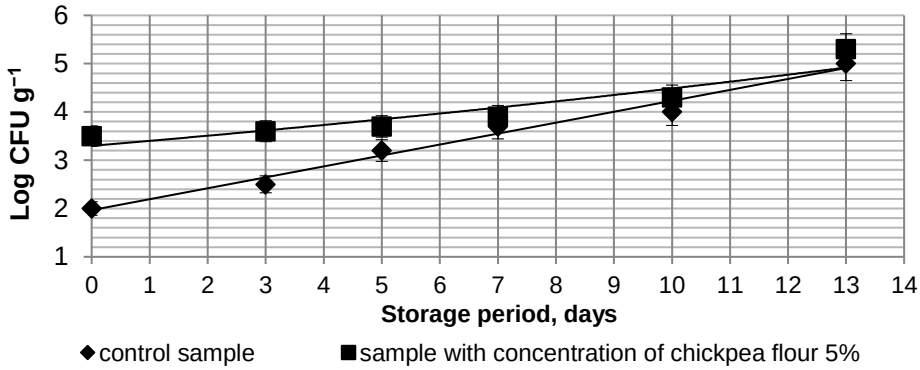

Figure 4. Microbiological changes in QMAFAnM during storage of cheese sample.

During the storage period, Escherichia coli bacteria, as well as potentially pathogenic and pathogenic microorganisms, fungus or yeasts were not detected in cheese samples.

Therefore, results of organoleptic, physical, chemical and microbiological analyses allowed to establish an expiration date of the product of 10 days under conditions of t = 4 ± 2 °C and relative humidity of 80 to 85%.

The output of each sample cheese from VAT milk (CY_v) was calculated using Eq. 1 (Heino et al., 2010). It is noted that with the increase of concentration of chickpea flour the product yield is also increased (Fig. 5), the difference between control sample and cheese product with chickpea flour yield is 12%. Hence, the product yield increases, at the same time gets functional properties, and is enriched with vegetable protein.

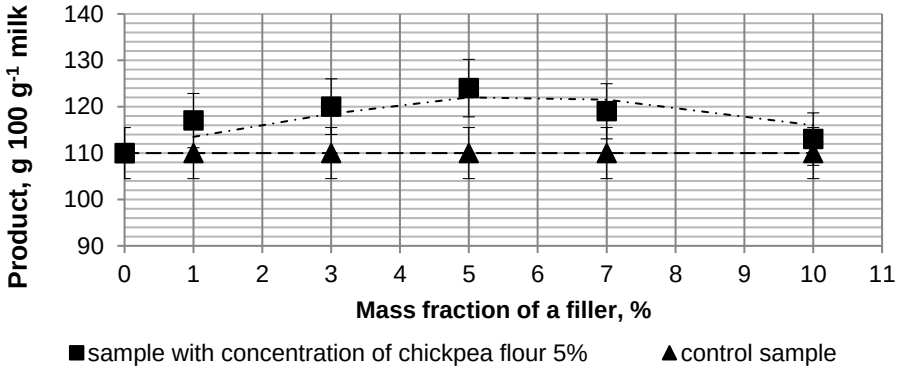

Figure 5. Effect of chickpea concentration on cheese yield.

It is well known that casein micelles in bulk milk are from 40 to 200 nm in size (Fig. 6, a), submicelle size is 12–20 nm, while the size of casein molecules of which submicelles consist of, as well as whey proteins, is in the range between 3 and 6 nm.

Fig. 6 shows the electron microscopic image of the rennet curd structure with the addition of chickpea flour.

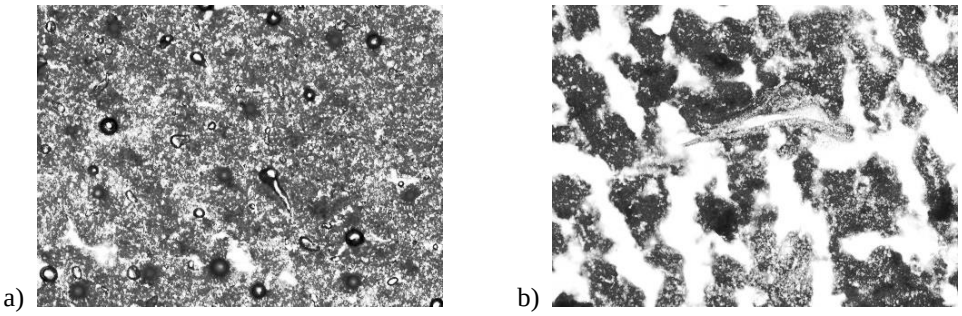

Figure 6. a) Histological slice of the product; b) Histological slice of the product with addition of chickpea flour in the amount of 5%.

During the experimental research, it was discovered that particles of chickpea flour slightly retard the process of gel syneresis; partially block the influence of the ferment on kappa-casein; physical and chemical processes of enzymatic formation of milk gel with flour particles run differently than with calcium chloride; particles of chickpea flour contribute to the binding of whey protein; it is probable that particles of chickpea flour also contribute to the binding of lactose.

CONCLUSIONS

Goats produce only approximately 2% of the world total annual milk supply. Goat milk differs from cow milk due its higher digestibility, alkalinity, buffering capacity, and certain nutritional and therapeutic properties.

A possibility of using chickpea flour in the soft cheese production was scientifically substantiated and proven practically, which allows enrichment of the product with vegetable protein and also increase product yield as well as to expand the assortment of dairy food products.

The optimal level of chickpea flour that maintains high technological properties of the cheese, consumer properties and nutritional value of the product was determined. The desired level of chickpea flour is 5% of weight of the mixture.

It is established that the introduction of chickpea flour into the soft cheese product has a positive influence on its organoleptic evaluation, vastly improves its nutritional and physiological values by means of enrichment with physiologically functional ingredients: phospholipids, polyunsaturated fatty acids, dietary fibers, vitamins, macro- and microelements, contributes to an increase in water retention capability of the product, increases product yield by 12% comparing to the control sample.

In addition, considering the almost complete absence of scientific works on the production of such cheeses, the obtained results could give a greater understanding of the characteristics of these products.

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Identification of kinetics parameters of wheat straw and sugar beet pulp hydrolysis with sulphurous acid

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Abstract. The mathematical modeling of the conversion of hemicelluloses of wheat straw and sugar beet pulp into monosaccharides using diluted sulphurous acid (0.59 and 1.18% wt) at high temperatures was performed. Kinetic equation, activation energy, pre-exponential factor and reaction order of the acid catalyst were determined. It was shown that agreement between the experimental data and kinetic model is good. It was proved that the process proceeds in the kinetic region and the entire array of measurements of the yield of monosaccharides satisfactorily described by the model with the minimum number of steps. The model predicts a decrease of duration of the process and increase of monosaccharides concentration in hydrolysates of lignocellulosic feedstock with temperature increase.

Key words: dilute acid hydrolysis, hemicelluloses, sulphurous acid, kinetic parameters.

INTRODUCTION

Conversion of plant's polysaccharides to monosaccharides solves important problems of processing and utilization of agricultural wastes and promotes the production of new products for chemical industry and biotechnology. Usually processing of plant biomass into bioethanol includes following stages: pretreatment, enzymatic hydrolysis, fermentation. Due to the fact that plant biomass can vary in composition (lignin amount, composition of hemicelluloses), each stage can be complicated and include diversified conditions (Donkoh et al., 2012). Stages of pretreatment and enzymatic hydrolysis have the greatest commercial cost (Wooley et al., 1999). Fermentable carbohydrates arise as a result of these two stages. In the event that lignocellulosic residuals are subjected to enzymatic hydrolysis without pretreatment obtained complex mixture of carbohydrates will be fermented not effective and hydrolysis will take a long time (Scheper et al., 1999). Pretreatment of plant raw materials, which enables removal of substances hindering the enzymatic hydrolysis, can have various rates of aggressiveness (Alvira et al., 2010). This treatment must be intensified to break chains of cellulose and hemicelluloses (Scheper et al., 1999); however, too much aggressive treatment can lead to formation of non-hydrolyzable products, as well as toxic for yeast products – furfural and hydroxymethylfurfural (Palmqvist & Hahn-Hägerdal, 2000). Acid hydrolysis is one of the most promising pretreatment methods with respect to industrial implementation Jönsson & Martín, 2016; Germec et al., 2016).

In spite of high catalytic efficiency of sulphuric, hydrochloric and phosphorus acid, problems of neutralizing and regeneration of these acids remains unsolved. Higher efficiency of sulphurous acid relative to sulphuric acid during pretreatment of plant raw materials was shown as exemplified by wheat straw (Nurtdinov et al., 2011). Besides, partial regeneration of sulphurous acid after pretreatment completion is possible through absorption of sulphur dioxide distilling stream.

Wheat straw and sugar beet pulp were used as sources of biomass for this study. These agricultural lignocellulosic residuals are renewable, cheap, and abundant wastes in Russia. High content of easily hydrolyzable polysaccharides in these raw materials stipulates expediency of its using for the production of commercially significant products such as furfural and its derivates, feed additives and food.

Predictive modeling of monosaccharides yield during agricultural raw materials acid hydrolysis is attractive for further development of technological processes and equipment.

Several kinetic studies of wheat straw and sugar beet pulp acid hydrolysis with sulphuric, hydrochloric, maleic and phosphorus acids have been reported in the literature (Ranganathan et al., 1985; Gonzalez et al., 1986; Jiménez & Ferrer, 1991; Tsoutsos & Dimitris, 2011; El-Tayeb et al., 2012; Katsamas & Sidiras, 2013). Significant discrepancies in values of the kinetics parameters obtained by various authors can be explained by differences in the applicable form of raw materials, the type and concentration of the hydrolyzing agent pretreatment conditions, hydrolyzing temperature, solid-to-liquid ratio and employed models and reactors.

Since conditions of hydrolysis and variations in composition and structure of biomass affect reaction rates, it is necessary to know the kinetic parameters that determine optimal conditions of treatment. The goal of this study was approximate evaluation of kinetics parameters hydrolysis of abundant wastes products using diluted sulphurous acid.

MATERIALS AND METHODS

Wet sugar beet pulp (*Beta vulgaris)*, produced on LLC ‘Buinsk sugar refinery’ (Buinsk city, Russian Federation; February, 2013) and wheat straw were used as raw material in this work. Moisture content of sugar beet pulp was 80.3 ± 0.3%, giving solid to liquid ratio close to 1:4. To obtain the lower solid to liquid ratio (1:3), dried sugar beet pulp with moisture content 2.0 ± 0.3% was added in corresponding amounts. Solid to liquid ratio was calculated as ratio of absolutely dry matter amount (g) of sugar beet pulp to total quantity of water including moisture (g) contained in feedstock. Wet samples were stored in freezing chamber at -6 °C.

Moisture content of heat straw was 2.2 ± 0.2%.

To determine dry-matter content, samples (2.0 g) were weighed into porcelain cups (diameter 50 mm) and dried in an oven at 105 °C for up to 3 days, until the weight was constant.

Composition analysis of easily-hydrolysable polysaccharides and hardly-hydrolysable polysaccharides

For determination of easily-hydrolysable polysaccharides sample of sugar beet pulp was hydrolysed with 2 M trifluoroacetic acid at 120 °C during 1 h. For determining of hardly-hydrolysable polysaccharides the remaining residue was hydrolysed with 72% sulphuric acid during 1 h at 25 °C, then water was added to achieve 3.4% final concentration of sulphuric acid and the sample was kept at 120 °C during 1 h. The obtained solution was neutralized with barium hydroxide (Fry, 1988). Further, the monosaccharide content and composition of the clarified hydrolysates was determined (Kharina et al., 2014; Kharina et al., 2016).

Conditions of pretreatment with sulphurous acid

Hydrolysis of sugar beet pulp was carried out in sealed thermostatically controlled capsules in a laboratory bench unit of proprietary design (Nurtdinov et al., 2011). The parameters of the hydrolysis process are represented in the Table 1.

Table 1. Regimes of the hydrolysis process

Raw material	T (°C)	C_{ac}, (wt %)	S/L*
Wheat straw	180–190	0.59; 1.18	1:5.8
Sugar beet pulp	180–190	0.59; 1.00	1:3

*S/L – solid- to- liquid ratio.

To check the course of hydrolysis process, the sampling was carried out each 5 min from 0 to 50 min of treatment. Experiments were conducted three times.

Analysis of oligosaccharides and monosaccharides

For separation of polysaccharides and low molecular weight carbohydrates (mono- and oligosaccharides) released during hydrolysis of sugar beet pulp, 96% ethanol was added to clarified hydrolysates to reach the final concentration of 80%. High-molecular weight polymers (polysaccharides) precipitated in ethanol, while low molecular weight carbohydrates remained in supernatant. Content of polysaccharides in hydrolysates was determined by the weight of the pellet which was formed upon treatment with ethanol after drying it.

Quantitative and qualitative composition of free monosaccharides in aliquates of supernatant was analyzed by means of high performance anion exchange chromatography on a column CarboPac PA-1 (4 x 250 mm, Dionex, USA) using a pulsed amperometric detector (Dionex). Elution rate was 1 ml min^{-1}, column temperature 30 °C. Buffers: A–100 mM NaOH in 1 M NaOAc, B–15 mM NaOH. Gradient elution was carried out as follows: 0–20 min B–100%; 20–21 min B–90%, A–10%; 22–41 min B–50%, A–50%; 42–55 min A–100%; 56–85 min B–100%. Results were analyzed using PeakNet software (Kharina et al., 2014; Kharina et al., 2016).

In order to determine oligosaccharides, the rest of supernatant obtained after ethanol precipitation of polysaccharides was dried and hydrolyzed with 2 M trifluoroacetic acid during 1 h at 120 °C. Quantitative and qualitative composition of monosaccharides after hydrolysis was estimated as mentioned above. Composition of oligosaccharides was determined according to the difference of individual monosaccharide content before and after hydrolysis with trifluoroacetic acid.

Qualitative and quantitative composition of polysaccharides is presented in (Kharina et al., 2014; Kharina et al., 2016).

The kinetic models for hemicellulose hydrolysis

To describe the mechanism of polysaccharides hydrolysis different simplified formal kinetic models are used. A number of researchers suggest that a simple two-step pseudo-homogeneous model can be applied for this type of reaction (Saeman, 1945; Ranganathan et al., 1985; Gonzalez et al., 1986; Jacobsen & Wyman, 2000; Tellez-Luis et al., 2002; Karimi et al., 2006; Lenihan et al., 2010; Katsamas et al., 2013).

This model use pseudo-homogeneous irreversible first-order reactions

$$P \xrightarrow{k_1} M \xrightarrow{k_2} \textit{decomposition products} \tag{1}$$

where: P, M – polysaccharide and monosaccharide, respectively; k_1, k_2 – the reaction rate constants for generation and decomposition of monosaccharide, respectively (min^{-1}).

Different hemicelluloses of raw material in kinetic calculations were considered as separate polymers whose hydrolysis occurs independently. Based in these two reactions, the differential equations that describe the changes of concentrations of substances are as follows:

$$\frac{d[P]}{dt} = w1, \quad \frac{d[M]}{dt} = w2 \tag{2}$$

where:

$$w1 = -k_1 \cdot [P] \tag{3}$$

$$w2 = \mu\, k_1 \cdot [P] - k_2 \cdot [M] \tag{4}$$

The concentration of monosaccharides is determined by the equation:

$$[M] = \mu \cdot \frac{[P_0] \cdot k_1 \cdot}{(k_2 - k_1)} \cdot \left(e^{-k_1 \cdot t} - e^{-k_2 \cdot t} \right) + [M_0] \cdot e^{-k_2 \cdot t} \tag{5}$$

where: $[P_o]$ – initial concentration of polysaccharide, measured in a process of complete hydrolysis ($mol\ l^{-1}$); $[M_o]$ – initial concentration of monomer; $[M_o] = 0$; t – duration of hydrolysis (min); μ – stoichiometric conversion factor of polysaccharide to monosaccharide.

Reaction rates constants were calculated, using:

$$k = A \cdot e^{-Ea/(R \cdot T)} \cdot [C_a]^{\alpha} \tag{6}$$

where: A – pre-exponential factor (min^{-1}); E_a – activation energy ($kJ\ mole^{-1}$); T – temperature (K); C_a – acid concentration; R–universal gas constant ($kJ\ mole^{-1}\ K^{-1}$); α–exponential acid parameter.

The chosen values were the ones giving the minimum values of the Sum of Squares of Error (SSE) for run data. The minimization of errors was performed using the ODE Solver function for Mathcad. The SSE was defined as

$$SSE = \sum (C_{calc} - C_{\exp})^2 \rightarrow min \tag{7}$$

RESULTS AND DISCUSSION

Hydrolysis was carried out according to conditions reported in Materials and Methods. Parameters of wheat straw and sugar beet pulp hemicelluloses hydrolysis were determined based on experimental data using pseudo-homogeneous kinetic model.

Experimental data

The main part of the chemical composition of the studied samples of lignocellulosic feedstock is easily hydrolysable polysaccharides (Table 2). They are quite heterogeneous because can contain glucans, pentoses and organic acids e.g. galacturonic acid. So different hemicelluloses were chosen for the study due to the dissimilar biomass.

Table 2. Chemical composition of raw material (% dry matter)

	Easily hydrolysable polysaccharides		Difficult hydrolysable polysaccharides	
	Wheat straw	Sugar beet pulp	Wheat straw	Sugar beet pulp
Total	68.6	69.9	14.7	12.1
Xylose	36.5	4.6	0.4	0.5
Arabinose	10.4	16.6	0.2	0.4
Glucose	13.3	16.4	14.1	10.7
Galactose	3.8	8.3	traces	0.3
Rhamnose	1.8	6.6	0.0	0.1
Galacturonic acid	1.5	16.1	traces	0.1
Glucuronic acid	1.3	1.3	traces	0.0

Monosaccharides of wheat straw hydrolysates obtained during complete hydrolysis were represented mainly by xylose, arabinose and glucose due to the predominance of glucuronic arabinoxylan and glucan with mixed type of molecular bonds in easily hydrolysable polysaccharides. Hydrolysates of sugar beet pulp have high content of arabinose, galactose and low content of xylose due to the predominance of arabinans and arabinogalactans in the easily hydrolysable polysaccharides.

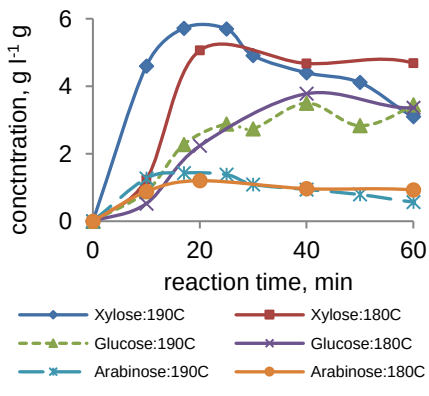


Figure 1. Kinetics of wheat straw hydrolysis (0.59%).

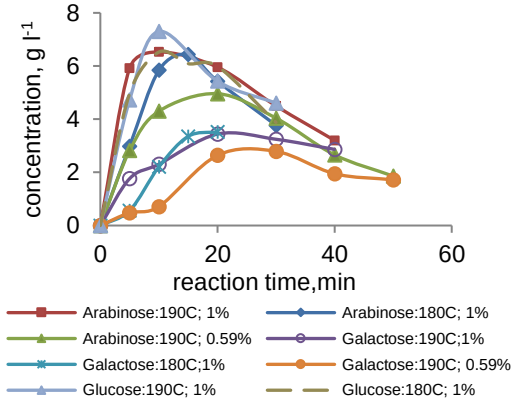


Figure 2. Kinetics of sugar beet pulp hydrolysis.

Hydrolysis of raw biomass with deluted sulphurous acid was carried out at 180–190 °C, maximum pretreatment time was 90 minutes. Experimental data of monosaccharides concentrations in hemicellulosic hydrolysates are represented in Figs 1 and 2. As known, during low-temperature hydrolysis (<160 °C) parallel firstorder reactions fast and slow fractions easily-hydrolysable polysaccharides hydrolysis can exist (Esteghlalian, 1997). As shown in Fig. 1 some difference in hydrolysis constants takes place for glucans and xylans at 180 °C. With increasing temperature reaction rate of these fractions are practically the same.

Intrinsic factors such as chemical constitution and location of hemicelluloses in the fibers of feedstock influence on hydrolysis rate. The time of achieving of maximum concentrations of glucose and arabinose slightly lower for sugar beet pulp then for wheat straw. Cell wall of sugar beet pulp less strong. Microfibrilles and hemicelluloses of sugar beet pulp submerged in matrix of pectic polysaccharides. Pectic polysaccharides consists of galacturonic acid residues linked α-(1→4)- bonds, and can include (1→4)-linked β- galactans with low degree of polymerization (Roberts, 1991; Carpita & Gibeaut, 1993; McCann & Spagnuolo et al., 1997).

Kinetic modeling of hemicelluloses hydrolysis

The effective reaction rate constants of hydrolysis with dilute sulfurous acid was obtained by curve fitting with the least sum of square error. Constants and the statistical coefficient R^2 derived from these study are displayed in Table 3.

Table 3. Rate constants

Process conditions		Constants		R^2
		$k_1(min^{-1})$	$k_2(min^{-1})$	
Wheat straw				
Arabinose	$C(H_2SO_3)$=0.59 wt %; 190°; S/L=1:5.8	0.0238	0.2207	0.82
Glucose		0.0078	0.0238	0.75
Xylose		0.0167	0.1338	0.83
Sugar beet pulp				
Arabinose	$C(H_2SO_3)$=1.00 wt %; 190°; S/L =1:3	0.0333	0.2125	0.82
Galactose		0.0152	0.0958	0.83
Glucose		0.0351	0.2124	0.70

The values of k_1 are higher for arabinose, it will be the first accumulation (Table 3). This trend persists at higher concentration (1.18% H_2SO_3; T = 190 °C; Arabinose: $k_1 = 0.0314\ min^{-1}$; Xylose: $k_1 = 0.0256\ min^{-1}$). The faster susceptibility of arabinose to acid hydrolysis is in line with literature data. In works (Dudkin & Gromov, 1991) it was noted that under equivalent conditions arabinose appeared in solution at a faster rate than xylose, presumably due the ease of cleaving of the single α1-2 or α1-3 arabinose linkages to the xylan backbone. Later during hydrolysis decomposition of xylans occurs with forming of dextrins and xylobioses.

In consequence of low catalytic activity of sulphurous acid obtained hydrolysis constants (k_1) are lower than hydrolysis constants for hydrolysis of wheat straw with hydrochloric and sulphuric acid. Nevertheless the values are consistent with the evidence for similar lignocellulosic materials (Lenihan et al., 2010). It ought to be noted that the value of monosaccharides decomposition reaction constant (k_2) is higher than hydrolysis

constant. Similar dependence during hemicelluloses hydrolysis was observed in other works (Carvalheiro, et al., 2005; Lenihan et al., 2010). Temperature extrapolation for k_1/k_2 of wheat straw is shown in Fig. 3. The value of constants ratio increases with temperature growth.

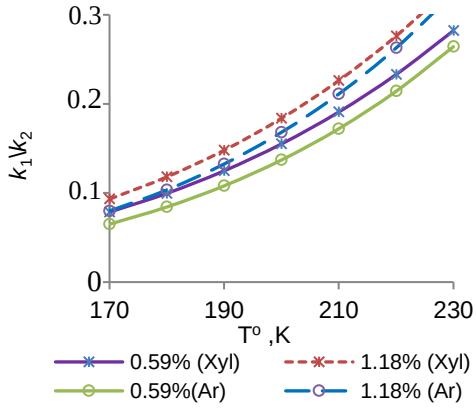


Figure 3. Dependence of k_1/k_2 of Xylose and Arabinose of Wheat straw.

In describing of hydrolysis using pseudohomogeneous kinetic model is necessary to ensure a в behavior of process in kinetic region. Kinetic region of heterogeneous processes are usually characterized by relatively high activation energy (E_a > 40 *kJ mole*$^{-1}$) and a strong dependence of the rate of reaction temperature (changing in 2–4 times for each 10 °C**)**. Based on the values of the activation energy (Table 3) and temperature coefficient of Van't Hoff (Table 4), we can conclude that at least for the wheat straw the process remains in the kinetic region.

Table 4. Temperature coefficients of the Van't Hoff (at 180–190 °C)

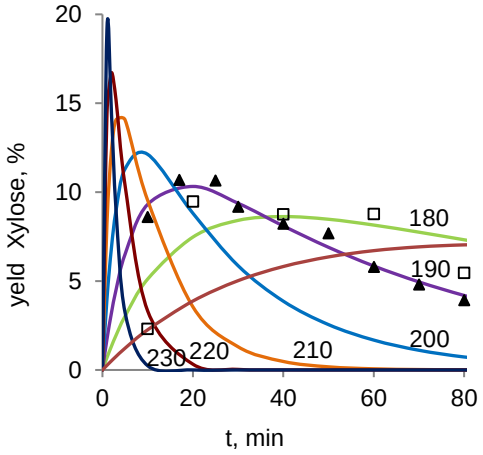
Raw material	Arabinose	Galactose	Glucose	Xylose
Wheat straw	2.65	-	1.91	2.60
Sugar beet pulp	1.85	1.60	1.44	-

The Arrhenius parameters and exponential acid parameters for different hemicelluloses represented in Table 5. The value of apparent activation energy hydrolysis (E_1) for Xylan → Xylose coincides with literature value, which obtained for the hydrolysis of wheat straw by using sulfuric treatments: 167.0 *kJ mole*$^{-1}$ (Ranganathan et al., 1985). As is shown in Table 5, for WS and SBP the E_2 for degradation of monosaccharides is significantly higher than E_1. Values of the Arrhenius parameters of sugar beet pulp is rather lower for sugar beet pulp than for wheat straw.

Table 5. Kinetic parameters of the acid hydrolysis of lignocellulosic feedstock

	A_1 (min^{-1})	E_1 (kJ·mole^{-1})	α_1	A_2 (min^{-1})	E_2 (kJ mole^{-1})	α_2
	Wheat straw					
Arabinose	9.44E + 17	169.7	0.397	5.25E + 13	126.1	0.102
Glucose	2.49E + 12	112.4	1.592	2.77E + 14	129.8	1.243
Xylose	5.26E + 17	166.6	0.617	7.80E + 13	127.1	0.372
	Sugar beet pulp					
Arabinose	4.07E + 11	107.2	1.088	5.25E + 13	64.5	0.896
Galactose	4.64E + 08	81.5	1.407	3.34E + 08	75.4	1.127
Glucose	4.13 E + 06	63.4	1.006	1.77E + 04	37.9	0.802

The Arrhenius parameters allow to predict conversion reactions of wheat straw and sugar beet pulp under any combination of time, temperature and acid concentration. Figs 4, 5 show the comparison between the model predicted values and experimental values. As mentioned earlier, temperature dependence of speed of the process is defined with constants ratio (k_1/k_2), which increases with temperature (Fig. 3). Consequently with increasing temperature, the kinetic model predicts a decrease in the optimal time of the process for all types of feedstock, as well as an increase in the maximum concentration of monosaccharides. The concentration of the acid catalyst has a significant effect for wheat straw monosaccharides, whereas sugar beet pulp characterized by a weak dependence of coefficients of formation and decomposition from the concentration of sulfurous acid.



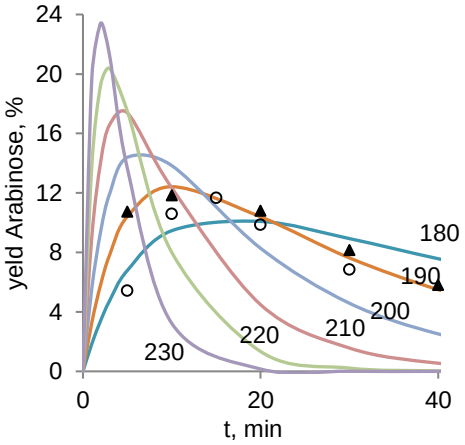


Figure 4. The yeld of Xylose of WS (0.59%): ○ – 180 °C; ▲ – 190 °C; ▬ – model.

Figure 5. The yeld of Arabinose of SBP (1%)○ – 180 °C; ▲ – 190 °C; ▬ – model.

As we can see from the Figs 4 and 5, empiric two-stage model, based on consecutive, irreversible reactions quasi-first order, shows good agreement between the experimental data and kinetic model.

CONCLUSIONS

The reaction of wheat straw and sugar beet pulp hemicellulose hydrolysis catalyzed with dilute sulphurous acid, was studied by kinetic modeling analysis. The effect of temperature, acid concentration and duration of hydrolysis on the efficiency of hydrolysis was investigated. Computational experiments were carried out, and kinetic parameters of the process (rate constants of hydrolysis, reaction rate constants for decomposition of monosaccharides, values of apparent activation energy, pre-exponential factor, exponential acid parameters) were determined, coefficients of determination were calculatec. It was shown that the process proceeds in the kinetic space and the entire array of measurements of the yield of monosaccharides satisfactorily described by the model with the minimum number of steps. Sulphurous acid is the less effective catalyst of hemicelluloses hydrolysis than sulphuric and hydrochloric acid. It

finds expression in lower constants of hydrolysis for sulfurous acid. Nevertheless it has advantage over other acids in the context of low cost, capability of regeneration and ecological safety. Maximum yields of xylose and arabinose lie in the range from 10 to 12%, depending on type of raw material. These yields can be obtained for the majority of hemicelluloses at reaction time lower than 20 minutes and more mild conditions (1.18% H_2SO_3, 190 °C). Supposedly, low yield can be explained with fast decomposition of monosaccharides, which can be confirmed with high values of corresponding reaction constants. It was shown that arabanes of wheat straw are more readily hydrolyzed than xylans. The values of apparent hydrolysis constants of wheat straw are sufficiently close to the typical values reported in the literature, but values of reaction rate constants for decomposition of monosaccharides are essentially higher. Values of hemicelluloses activation energy and arrhenius parameters for sugar beet pulp are essentially lower than for wheat straw. Good agreement of experimental and calculated kinetic data obtained using two-stage model of acid hydrolysis to be indicative of availability this approach for quantitative interpretation of experimental results.

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Sewage sludge composting and fate of pharmaceutical residues – recent studies in Estonia

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Abstract. This review is to reflect the work addressed to the application of biosolids and especially sewage sludge as a resource in composting. A considerable drop in the use of P fertilisers can be followed since early 1990s. Due to this fact crop production in Estonia takes place at the expense of soil phosphorous (P) resources. One of the ways of increasing the fertility of agricultural lands is to use nutrient-rich sewage sludge. Unfortunately, this may cause several undesired consequences due to biological and chemical contaminants. The presence of some widely used pharmaceuticals, as ciprofloxacin (CIP), norfloxacin (NOR), ofloxacin (OFL), sulfadimethoxine (SDM) and sulfamethoxazole (SMX), was evident in sewage sludge of the two Estonian largest cities, Tartu and Tallinn. The concentrations of pharmaceuticals decreased after sewage sludge digestion and composting, but they were still present in detectable amounts. Sewage sludge co-composting experiments with sawdust, peat and straw showed the degradation of fluoroquinolones (FQ) and sulfonamides (SA). Additions of sawdust clearly speeded up this process, whereas the mixtures with peat and straw performed lower abilities to decompose pharmaceutical residues. Novel methodologies were developed and experiments conducted to study the potential accumulation of fluoroquinolones FQs and SAs by food plants. Due to the low adsorption of SAs on soil particles they are 'free' to migrate into plants. Different behaviour is characteristic to FQs as they are accumulated in sludge. Recent years have also shown progress in vermicomposting work and in using compost in afforestation.

Key words: composting technologies, fertilizers, pharmaceuticals, plant uptake, sewage sludge, vermicomposting.

INTRODUCTION

Land application of biosolids is generally considered to be the best option of disposal because it offers the possibility of recycling nutrients, provides organic material, improves soil properties, and enhances crop yields (White et al., 2011). Higher soil quality is generally associated with higher concentrations of soil organic matter and a plentiful supply of essential elements. Thus, the recycling of organic matter from anthropogenic residues to soil often benefits agricultural sustainability (White et al., 2013). However, this benefit has to be weighed against potential deleterious effects (White et al., 2011). Whilst recognising its significant value as a resource, recycling

sewage sludge to agricultural land requires a careful management to avoid potential negative impacts on the environment from chemical contaminants (Torri et al., 2012).

Organic residues recycling via composting appears to be an ancient activity. The practice of converting animal manure and other biodegradable wastes to compost is believed to have originated as early as agriculture (Fitzpatrick et al., 2005). The earliest known written reference to composting is found in clay tablets dated to the Akkadian empire, about 4,300 years ago (Rodale, 1960), but it is believed that the fertilizer value of aerobically degraded organic matter, which we now call compost, was recognized much earlier. There is evidence that the Romans, Greeks, and the Bani Israel knew about compost. The Bible and Talmud both contain numerous references to the use of rotted manure straw, and mention of compost occurs in 10th and 12th century Arab writings, in medieval Church texts, and in Renaissance literature (Smith et al., 2007).

A worldwide massive use of biosolids as soil conditioners and fertilizers arose in the early 1900s (Frank, 1998). Increasing urbanization and industrialization have resulted in a dramatic growth in the amount of wastes generated globally, particularly of sewage sludge as a byproduct from sewage treatment (White et al., 2011). Land application of treated sewage sludge and other biosolids improves soil fertility and has an important role in closing nutrient cycles (Torri et al., 2012). Among the macronutrients contained in sludge, phosphorus is an essential element for plant metabolism, often considered one of the most limiting nutrients for plant productivity (Shaheen et al., 2012).

A large variety of plant, animal and synthetic wastes can be gainfully composted at scales varying from a household bin to a large industry (Gajalakshmi & Abbasi, 2008). In the composting process, aerobic microorganisms use organic matter as a substrate (Gajalakshmi & Abbasi, 2008). The microorganisms decompose the substrate, breaking it down to more simple compounds (Epstein, 1997; Ipek et al., 2002). During composting, carbon- and nitrogen-containing compounds are transformed through successive activities of different microbes to more stable organic matter, which resembles humic substances (Pare et al., 1998). The rate and extent of these transformations depend on available substrates and the process variables used to control composting (Marche et al., 2003; Gajalakshmi & Abbasi, 2008).

Inventories of soil productive capacity indicate human-induced soil degradation on nearly 40% of the world's arable land (Doran & Zeiss, 2000); this warns us of the ecological collapse of the world's productive soils (Pankhurst et al., 1997). In Estonia the highly industrialised and centralised agricultural production system collapsed in the late 1980s and early 1990s. The area of arable land (crop fields and cultural grasslands) decreased from about one million ha in the early 1990s to less than 0.6 million ha by 2003 (Statistics Estonia, 2006; Iital et al., 2014). Also, a considerable drop in the use of N and P fertilisers took place in the early 1990s when it constituted only about 13% of the peak in 1987–1988. Based on the data from Statistics Estonia in 1994–2001 the average annual consumption of commercial fertilisers was only 85 kg ha^{-1} and in 2009–2011 it reached the level of 120 kg ha^{-1} (Statistics Estonia, 2012; Iital et al., 2014). Since mid–1990s the national average soil P balance has been negative in Estonia due to a sharp decrease in fertilizer use and availability of manure. The national average soil P balance varied in 2004–2009 from -10 to -5 kg P ha^{-1}. Currently crop production in Estonia largely takes place at the expense of soil P resources (Astover & Rossner, 2013).

One of the most efficient ways to eliminate this problem is an intelligent usage of solid waste composts.

This overview is to reflect recent research performed mainly in Estonia in the area of composting. These studies involved different aspects of sewage sludge composting and compost usage; vermicomposting of different waste materials; possible undesired consequences associated with the application of composts in agriculture.

NECESSITY FOR COMPOSTING AND RESOURCES (BACKGROUND)

The soil cover of Estonia is relatively varied due to the alternation of carbonate and humus-rich soils with acid soils which are relatively poor in nutrients and organic matter (Köster & Kõlli, 2013). The lack of nutrients is especially obvious in the case of peatlands which cover 22.3% (10,091 km^2) of Estonia's territory, so restricting the usage of these lands for agricultural purposes. The awareness of the composition and properties of soil cover and its relationship with plant cover in different land use conditions is the basis of ecologically proper and sustainable management of land and soil resource (Köster & Kõlli, 2013).

Estonia has the world's largest exploited oil-shale basin covering about 4% of its territory. In 2001−2013 the number of active landfills in Estonia decreased from 159 to 13. Recultivation of the landscapes covered by semi-coke, oil-shale ash mountains, abandoned opencast mines and closed landfills appears to be one of the major environmental tasks in Estonia.

Biosolids can be used in biofuel production (Raud et al., 2014), leading to the incineration of organic matter. Perceived as a green energy source, the combustion of biosolids has received renewed interest. Still, anaerobic digestion is generally a more effective method than incineration for energy recovery, and digested biosolids are suitable for further beneficial use through land application (Wang et al., 2008). The use of biosolids as a source of organic matter may improve the physical and chemical properties of agricultural soils resulting in an increase in crop yields (Torri et al., 2014). The major potential source for making compost in Estonia is sewage sludge. The yearly generation of sewage sludge by Estonian sewage treatment plants is 30,000 tonnes dw.

Semi-coke is the waste product of oil shale industry and presents the hazard to the environment, due to its phenol and PAH content. One of the main problems of oil shale industry is how to treat semi-coke effectively (Wang et al., 2009). In 1993−2003 the volume of semi-coke formed in Estonian shale oil enterprises varied within 0.6 and 1.4 million tonnes annually (Pae et al., 2005). It has been established that the compost made from semicoke and sewage sludge increases the yield of the crops (Varnik et al., 2006).

The average quantity of biodegradable waste generation in Estonia from grocery stores during 2004–2010 was 9 thousand tonnes $year^{-1}$. The results of SWOT analysis published by Blonskaja et al. in 2014 showed that composting process is the best solution for kitchen wastes. It has been demonstrated that one of the ecologically and environmentally friendly alternatives to traditional technologies in organic wastes management is vermicomposting, especially in kitchen wastes treatment (Ivask et al., 2013; Peda & Kutti, 2013; Haiba et al., 2014; Sinha et al., 2014).

SEWAGE SLUDGE COMPOSTING AND ENVIRONMENTAL CONCERNS

Unprecedented growth in urban population has resulted in the generation of huge quantities of wastewater worldwide (Singh & Agrawal, 2010). Wastewater treatment facilities are responsible for treating large volumes of domestic and industrial sewage containing human waste. The treatment goal is to produce effluents of high enough quality for discharge back into the environment. Sewage sludge is a byproduct of this process and necessitates proper disposal (Walters et al., 2010; Zuloaga et al., 2012). Safe disposal of sewage sludge is one of the major environmental concerns (Singh & Agrawal, 2010).

Historically, sewage sludge has been disposed of by incineration, landfilling or ocean disposal (Bridle & Skrypski-Mantele, 2000). Nowadays, the most widespread method for sewage sludge disposal has become agricultural application, since it is the most economical outlet for sludge compared to incineration and landfilling (Zuloaga et al., 2012; Li et al., 2013; Chen et al., 2014). The use of sewage sludge in agriculture is one of the major causes of environmental pollution (Nouri et al., 2008). Although, sewage sludge and its compost offers an opportunity to recycle plant nutrients and organic matter to soil for crop production stimulating biological activity (Rodríguez et al., 2012; Zuloaga et al., 2012; Li et al., 2013; Haiba et al., 2014), its usage as a fertilizer is limited due to a large number of toxic pollutants found in this matter (Lillenberg et al., 2010a; Lillenberg, 2011).

Composting is recognized as one of the most important recycling options for sewage sludge (Hara & Mino, 2008; Dorival-García et al., 2015). Since sewage sludge is mainly composted in Estonia and often re-used in agriculture as a fertilizer, several composting methods are applicable, but the selection of the method is dependent on the investment and operation cost, time required to reach compost stability and maturity, the availability of land, origin of raw materials and bulking agents (Ruggieri et al., 2008; Mollazadeh, 2014; Nei et al., 2014).

Several sludge composting experiences have been shared in Estonia (Kanal & Kuldkepp, 1993; Varnik et al., 2006; Kriipsalu et al., 2008; Kriipsalu & Nammari, 2010; Lillenberg et al., 2010a; Holm & Heinsoo, 2013; Kuusik et al., 2014; Menert et al., 2014). The most common sewage sludge composting methods are: static piles, aerated static piles, windrow and in-vessel systems (Yue et al., 2008). There are many factors that affect the composting process, such as the proportions of the mixture, temperature, rate of aeration, oxygen consumption rates, compost pile size, moisture content, pH and carbon-to-nitrogen ratio (Luo et al., 2008; Chen et al., 2014; Malinska et al., 2014; Nayak & Kalamdhad, 2014). Also, microorganisms play a key role in composting processes and nutrient turnover, and even slight changes in microbial activity and community composition due to antimicrobial agents may result in poor compost quality and prolonged time needed for compost stability (Nei et al., 2014). Respiration is a global measure of the total microbial activity that can provide a reliable, repeatable and scientifically sound assessment of microbial activity, respirometry (CO_2 evolution rate and/or O_2 uptake rate) has been widely used to evaluate microbial activity and composting efficiency (Liang et al., 2003; Barrena Gómez et al., 2006). The second widely used parameter for the evaluation of microbial activity is microbial biomass-C, measured by the substrate induced respiration based on Platen & Wirtz, 1999. Also, one of the methods of obtaining information about the dynamics of composting processes is

the bacterial-to-fungal ratio (Joergensen & Wichern, 2008). The microbial community may reflect the evolution and performance of the composting process thus acting as an indicator of compost maturity (Nei et al., 2014; Wang et al., 2015).

Since sewage sludge has high moisture content it cannot be composted alone − in order to absorb moisture it should be mixed with dry materials, which act as bulking agent thereby improving the aeration and the compost quality (Nayak & Kalamdhad, 2014; Zhou et al., 2014). Sludge and bulking agent proportions in compost influence the composting reaction rate and the final compost quality. Sludge can be mixed with different bulking agents, sources of carbon, such as peat, straw, wood chips, leaves, ash, peat, sawdust (Komilis et al., 2011; Cukjati et al., 2012; Maulini-Duran et al., 2013; Malinska et al., 2014).

A range of studies has shown that some pharmaceuticals and personal care products (PPCPs) are neither completely removed by sewage treatment, nor completely degraded in the environment (Redshaw et al., 2008; Lillenberg et al., 2009; Lillenberg et al., 2010a; Jelic et al., 2011; Rodríguez-Rodríguez et al., 2012; Borgman & Chefetz, 2013; Haiba et al., 2013b; Narumiya et al., 2013; Reichel et al., 2013). Although, their concentrations are much lower than the levels of traditionally known organic pollutants, the potential long-term effects of these compounds to humans, plants and animals cannot be ignored (Lillenberg et al., 2009; Nei et al., 2014; Van Doorslaer et al., 2014; Prosser & Sibley, 2015; Bártíková et al., 2016).

FATE OF PHARMACEUTICAL RESIDUES DURING SEWAGE SLUDGE COMPOSTING

Pharmaceuticals have been used for decades to prevent and treat human and animal diseases (Zhang et al., 2008; Li et al., 2014). Recently, there has been increasing concern about the effects of pharmaceuticals in aquatic and terrestrial ecosystems, as they can affect the efficiency of microbial-mediated processes (the regeneration of nutrients, carbon and nitrogen circulation and digestion of pollutants) in the environment (Girardi et al., 2011; Jelic et al., 2011; Bergersen et al., 2012; Martín et al., 2012; Chen et al., 2013; Li et al., 2014).

As a result of regular industrial, agricultural and household activities, a variety of compounds enter into the environment, of which only a small percentage are studied for their toxicological effects on humans and the environment (Peysson & Vulliet, 2013). Approximately 4,000 drug substance is used in Europe (human and veterinary), of which may have responsive impact to the environment (Rodríguez-Rodríguez et al., 2011**)**. About 150 medical compounds are studied that have been found in the environment, but mostly in water samples (Rivera-Utrilla et al., 2013; Li et al., 2014). For example, the Estonian Statistics on Medicines data show that over the years the proportion of consumption of different drugs has increased, both over-the-counter as well as prescription drugs (State Agency of Medicines, 2011; 2013). There is no reliable information of how many people actually do or do not consume their drugs, how many medicines are not administered and how many different compounds are thrown into the sewage system or to the garbage. The increasing proportions of administered drugs and personal care products is alarming because of the compound releases to the environment are not controlled (Motoyama et al., 2011; Gonzalez-Martinez et al., 2014), which

involves a potential threat to the environment (Vasskog et al., 2009; Rodríguez-Rodríguez et al., 2011; Peysson & Vulliet, 2013).

A wide variety of pharmaceutically active compounds are present in wastewater effluents, surface waters, and ground waters (GWRC, 2008), and the sewage treatment plants are unable to remove all these substances. The removal rates of individual drugs during passage through a sewage treatment plant have varied from 12 to 90% (Stumpf et al., 1999; Butkovskyi et al., 2016). The fate of pharmaceuticals may be divided into three principal routes (Richardson & Bowron, 1985):

1. The substance is ultimately mineralized to carbon dioxide and water;
2. The substance is lipophilic and not readily degradable, so part of the substance will be retained in the sludge. These substances are able to contaminate soil if the sludge is dispersed onto fields;
3. The substance is metabolised to a more hydrophilic form of the parent lipophilic substance, but is still persistent and therefore will pass the sewage treatment plant, ends up in the receiving waters (rivers, seas) and may therefore affect the aquatic organisms, if the metabolites are biologically active.

Presence of different pharmaceuticals in sewage sludge is apparent, but there is still a lack of information concerning the fate of pharmaceutical residues in the environment (Kümmerer, 2008; Lillenberg, 2011). Pharmaceuticals are often not readily degradable (Richardson & Bowron, 1985; Gavalchin & Katz, 1994; Marengo et al., 1997; Halling-Sørensen et al., 2002; Hamscher et al., 2002; Carballa et al., 2004). Still, remarkable amounts of pharmaceuticals enter the soil via fertilizing with sewage sludge (Golet et al., 2002; Haiba et al., 2013a).

Medical substances have many necessary properties to bio-accumulate and provoke change in ecosystems (Kipper et al., 2010; Baran et al., 2011). No trigger values exist for drug residues in sewage sludge neither in Estonia (Decree of Estonian Minister of the Environment) nor in the European Union (EU Council Directive 86/278/EEC; Lillenberg et al., 2009). The most closely related act is the EU directive EMEA/CVMP/055 establishing trigger values for drug residues in manure (EMEA/CVMP/055/96). The content of drug residues should not exceed 100 μg kg^{-1} in manure and 10 μg kg^{-1} in the soil fertilized with manure. Montforts (2005) suggests that these figures should be remarkably lower. Soil organisms, microflora and plants are directly exposed to contaminants in sludge-amended soils.

The presence and content of some widely used pharmaceuticals was determined in sewage sludge and in its compost in the two Estonian largest cities, Tartu and Tallinn (Lillenberg, 2011). The sewage sludge in Tartu was treated by composting – mixing with tree bark (volume ratio 1:1). The methane fermentation and mixing with peat (volume ratio 1:0.75) were used in Tallinn. The samples were taken from anaerobically digested sludge (before mixing with peat) in Tallinn and from untreated sludge (before composting) in Tartu. The concentrations of most of the pharmaceuticals (ciprofloxacin-CIP, norfloxacin-NOR, ofloxacin-OFL, sulfadimethoxine-SDM and sulfamethoxazole-SMX) decreased significantly after sewage sludge digestion and compost processes, but many of them were still present in compost. The degradation of pharmaceutical residues was more efficient in Tallinn probably due to anaerobic sludge digestion (compost was made by mixing the treated sewage sludge with peat) compared to the results obtained in Tartu (raw sewage sludge was mixed with tree bark). The results of the relevant pilot studies are described in detail in Lillenberg et al. (2010a) and Lillenberg (2011).

Interestingly, SDM was present in most sludge and in some compost samples, although this antimicrobial was not marketed any more during the years of 2007 and 2008 in Estonia. It is possible that 'old' supplies were put to use or small amounts of this chemical were imported from other countries (Lillenberg et al., 2010a; Nei et al., 2010).

According to Lillenberg (2011) the highest concentrations of pharmaceuticals were found in Tallinn sewage sludge: CIP 1,520 μg kg^{-1} and NOR 580 μg kg^{-1} (dm). The highest detected concentration of CIP exceeded the trigger value for manure (100 μg kg^{-1}) over four times. The concentrations of OFL (134 μg kg^{-1}), SDM (73 μg kg^{-1}) and SMX (22 μg kg^{-1}) were lower (Table 1). The average contents of antibiotics were: CIP 737 μg kg^{-1}, NOR 279 μg kg^{-1}, OFL 80 μg kg^{-1}, SDM 2 μg kg^{-1} and SMX 18 μg kg^{-1} (dm). As a rule, the concentrations of pharmaceuticals in Tallinn sewage sludge from were relatively low. Still, in some cases the concentrations of CIP, NOR and OFL were over the trigger value (Table 1).

Table 1. The highest concentrations of pharmaceuticals detected from Tallinn sewage sludge, μg kg^{-1} (dm) (reproduced from Lillenberg, 2011)

Month	CIP	NOR	OFL	SDM	SMX
January	**1,520**	**580**	**134**	3	22
February	67	67	17	73	5
March	58	31	8	3	1
April	58	33	3	n.d.	2
May	**150**	**215**	7	0.4	n.d.
June	**206**	**163**	17	n.d.	4
July	39	37	4	n.d.	n.d.
August	11	26	5	n.d.	4
September	0.4	0.4	n.d.	n.d.	n.d.
November	42	16	9	3	3
December	53	85	37	4	7

CIP – ciprofloxacin; NOR – norfloxacin; OFL – ofloxacin; SDM – sulfadimethoxine; SMX – sulfamethoxazole; n.d. – not detected.

In Tartu, contrarily, the concentrations of CIP and NOR were in most cases over the trigger value, the high content of OFL was detected only in August, September and October (Lillenberg, 2011). The content of sulfonamides (SAs – SDM and SMX) was quite low in both cities, under the trigger value set for drug residues in manure (100 μg kg^{-1}) (Tables 1, 2). In Tartu at least one of SAs was present in every sludge sample (Table 2). The contents of SMX were in the range of 0.0–22 μg kg^{-1}, and SDM 0.00–73 μg kg^{-1} (dm) in Tallinn. In Tartu contents of SMX were between 0.0–11 μg kg^{-1}, and SDM 0.0–32 μg kg^{-1} (dm). The highest concentrations of antimicrobials in sewage sludge from Tartu were: NOR – 439 μg kg^{-1} and CIP – 442 μg kg^{-1} (dm). OFL was present in every sludge sample from Tartu and the highest concentration was 157 μg kg^{-1} (dm) (Table 2).

Table 2. The highest concentrations of pharmaceuticals determined from Tartu sewage sludge, μg kg^{-1} (dm) (reproduced from Lillenberg, 2011)

Month	CIP	NOR	OFL	SDM	SMX
January	**315**	82	86	8	6
February	**423**	**263**	68	32	7
March	89	60	26	0.4	1
May	**174**	**264**	22	1	n.d.
June	**265**	**264**	47	n.d.	16
July	67	**104**	19	n.d.	6
August	**442**	**439**	**111**	24	n.d.
September	**231**	**188**	**157**	22	9
October	**259**	**126**	**149**	4	n.d.
November	**134**	**105**	33	6	11
December	71	40	32	9	6

CIP – ciprofloxacin; NOR – norfloxacin; OFL – ofloxacin; SDM – sulfadimethoxine; SMX – sulfamethoxazole; n.d. – not detected.

The degradation of pharmaceuticals was more efficient in the case of composting in Tallinn. During 12 months composting period the concentrations of all the studied pharmaceuticals diminished for 99.9%, whereas in Tartu this indicator showed the value on average 90 ± 4%. The only exception was SDM, which 'disappeared' fully in both cases. In Tallinn the anaerobically digested sludge was mixed with peat and composted. In Tartu raw sewage sludge was mixed with tree bark (1:1) and settled in piles. The media vas mixed at least twice per month during eight-months period. It has been shown, that a higher decrease of pharmaceuticals is observed after anaerobic digestion than after aerobic digestion, which can be explained by a higher degradation under anaerobic conditions (Martin et al., 2015).

The degradation rate of pharmaceutical residues is dependent on the initial components of the compost. Fine sawdust appears to be an excellent sewage sludge amendment: from the agricultural point of view, sludge co-composted with particularly fine-textured sawdust is claimed to be an excellent compost material to be applied to soils (Ammari et al., 2012; Nei et al., 2015). Kim et al. (2012) have shown that sawdust is able to initiate efficient composting, leading to elevated composting temperatures, and consequently resulting in the reduction of residual concentrations of pharmaceuticals to reasonable levels in a relatively short composting period.

According to Haiba et al. (2013b), composting remarkably reduces the concentrations of these pharmaceuticals. In most experiments their concentrations decreased by 95% or more during 4 months of composting (Table 3). The best results were obtained when the sludge was mixed with sawdust. In the case of using straw or peat instead the decomposition rates were lower. Additions of sawdust clearly speeded up this process, whereas the mixtures with peat and straw performed lower abilities to decompose pharmaceutical residues. No clear evidence was received concerning the impact of oil shale amendments on the degradation speed of the studied pharmaceuticals. Many studies have shown that sawdust has been proven to be a good bulking agent for sewage sludge composting (Banegas et al., 2007; Zorpas & Loizidou, 2008; Haiba et al., 2013a & 2013 b). The decline of tetracycline and sulfonamide concentrations was highly dependent on the presence of sawdust while there was no influence of sawdust on tylosin decline (Kim et al., 2012).

Table 3. Degradation of pharmaceuticals in sewage sludge compost mixtures during 4-months composting period, %

Bulking agent (% from dry matter)	**SMX**	**SDM**	**NOR**	**CIP**	**OFL**
1. peat (50)	83	77	90	92	100
2. sawdust (33)	100	99	96	95	100
3. sawdust + oil shale ash (29+14)	100	96	82	94	99
4. sawdust + wood chips (total 43)	100	99	91	98	86
5. straw (50)	99	98	79	90	74

CIP – ciprofloxacin; NOR – norfloxacin; OFL – ofloxacin; SDM – sulfadimethoxine; SMX – sulfamethoxazole.

PHARMACEUTICALS AND PLANT UPTAKE

The significance of the route involving the uptake of several medicines from soil by plants in terms of risk to human health is evident (Lillenberg et al., 2010b; Prosser & Sibley, 2015; Wu et al., 2015). As the compost made from sewage sludge contains detectable amounts of pharmaceutical residues, experiments were conducted to study the significance of their uptake into plants from soil under 'real' conditions. Therefore, experiments were performed to investigate the potential accumulation of the studied pharmaceuticals – fluoroquinolones (FQs) and sulfonamides (SAs) – taken up by food plants (namely – carrot, potato, lettuce, wheat) from the soil fertilized with sewage sludge or its compost. The results of these experiments are shown in Lillenberg et al. (2010a; 2010b), Kipper et al. (2010) and Nei et al. (2010).

The uptake of pharmaceuticals by the studied food plants was noticeable. It has been shown that due to the low adsorption of SAs on soil particles they readily migrate into plants (Haiba et al., 2013a). Different behaviour is characteristic to FQs due to their sorption to sewage sludge and soil particles (Golet et al., 2003). Therefore, as a rule, the content of SAs in the plants was higher. The content of the studied pharmaceuticals was higher in plats cultivated in sandy soil (Lillenberg, 2011). In loamy soil the molecules of both SAs and FQs attach to clay particles reducing their uptake by plants. Fig. 1 is to illustrate the said. The amounts of FQs going into potato do not depend much on soil type. The application of sewage sludge compost as a fertilizer and the following uptake of pharmaceuticals by food plants may cause contamination of these plants (Haiba et al., 2013a).

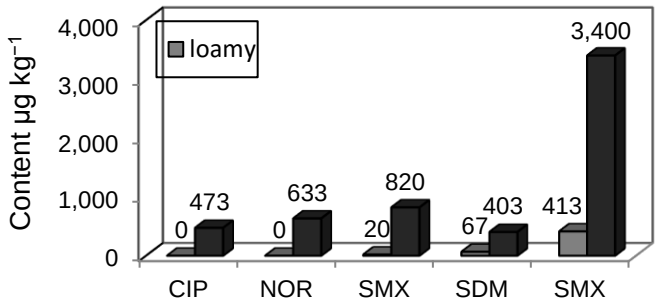

Figure 1. Average concentrations of pharmaceuticals in carrot roots grown in different soils at drug concentration of 10 mg kg^{-1}: CIP – ciprofloxacin; NOR – norfloxacin; OFL – ofloxacin; SDM – sulfadimethoxine; SMX – sulfamethoxazole.

Toxic compounds entering into the soil may affect microbial activity, plant growth and development and may have adverse effects on living organisms (Lillenberg et al., 2010b; Michelini et al., 2012; Haiba et al., 2013a; Nei et al., 2014). Further studies concerning the plant uptake of a wide spectrum of commonly used pharmaceuticals from soils fertilized with sewage sludge or its compost are needed to ensure food safety.

Lillenberg concludes in her PhD thesis (Lillenberg, 2011) that the residues of pharmaceuticals readily accumulate in several food plants. This phenomenon remarkably depends on the nature and concentration of a pharmaceutical and soil type. When using the sewage sludge compost as a fertilizer, it should be carefully tested for the safety. The content of pharmaceuticals in the compost made from sewage sludge may easily lead to the elevated concentrations in food plants if the compost is used as a fertilizer. Still, wheat grains had low or zero concentrations of the analysed pharmaceuticals. This confirmed the potential applicability of sewage sludge compost for fertilization of the crops of this type (Haiba et al., 2013a). Further work should be conducted to determine different types of pharmaceuticals and other organic pollutants by food plants (Lillenberg, 2011). It is evident that the development of novel sewage sludge treatment technologies are needed to solve environmental problems related to sewage sludge exploitation.

PUBLICATIONS AND THESES

Vermicomposting

Vermicomposting technology is a simple and environmentally friendly biological treatment of wastes. As a result of the work published in Ivask et al. (2013) and Haiba et al. (2014) the applicability and efficiency of using earthworms *Eisenia fetida* and *Dendrobaena veneta* in vermicomposting of sewage sludge and household organic residues in the countries with the climate comparable to Estonia was demonstrated.

Compost in afforestation

In Estonia the reforestation of depleted peat and sand mining areas is often complicated due to the unfavourable physical, chemical and biological properties of soils. The impact of artificial roots and soil amelioration with green waste compost in the afforestation of depleted peat fields and sand pits was studied. The results of this work is presented in Jarvis et al. (2012) and Jarvis et al. (2016). Added compost caused significantly improved height growth of the studies tree species seedlings, hence enhanced the growth conditions locally.

Development of novel methodologies for the determination of pharmaceutical residues

Novel approaches for the quantitative determination of traces of commonly used pharmaceuticals in sewage sludge and plants were developed (Lillenberg et al., 2009; Kipper et al., 2011; Kipper, 2012). The compounds were simultaneously extracted from sewage sludge by pressurized liquid extraction (PLE). A novel and effective method for PLE was developed. Solid-phase extraction was used for cleaning up the extracts.

Dissertations defended

PhD thesis: Karin Kipper, Fluoroalcohols as Components of LC-ESI-MS Eluents: Usage and Applications, 2012. A novel and efficient methodology for pharmaceutical analyses in complex matrices (e.g. blood plasma and environmental samples) was developed and tested.

PhD thesis: Merike Lillenberg, Residues of some pharmaceuticals in sewage sludge in Estonia, their stability in the environment and accumulation into food plants via fertilizing, 2011. The aim of the work was to study the presence of some widely used pharmaceuticals in Estonian sewage sludge and its compost and the uptake of these pharmaceuticals from fertilized soils by some food plants. As a result of this research the following was established:

1. Pharmaceuticals were present in sewage sludge and its compost from both Tallinn and Tartu and in several samples their concentrations exceeded the relevant trigger values for manure.
2. Degradation of pharmaceuticals took place as a result of composting.
3. The main reason of the decrease in pharmaceutical concentrations during composting was the applied sludge treatment technology.
4. The uptake of the studied pharmaceuticals by food plants was obvious. The application of sewage sludge compost as a fertilizer and the resulting uptake of pharmaceuticals by food plants may cause contamination of these plants.

CONCLUSIONS

Land application of composts is an important and efficient tool in the remediation of industrial landscapes and agricultural soils in Estonia. Still, due to the frequent presence of different undesired residues, composts made from sewage sludge need careful inspection before their use. The work should be continued by the development of novel and more efficient composting technologies, leading to intelligent solutions of environmental problems related to biowaste exploitation.

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Presentation title: Ability to handle unfamiliar systems in passenger cars according to driver skills

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Abstract. This paper addresses the ability of drivers to intuitively control special passenger car systems that they have as yet not encountered in the course of their driving practice and therefore have no experience of them. The study described in this paper was conducted on a sample group of drivers without any prior experience of the tested model or of any other model of the same brand, and the functions and systems selected for testing were unique for the brand and model in question. The reason for conduction of this study was the endeavour to recreate the common situation in which a driver is forced to drive a car with whose controls he/she has not yet had the opportunity to become acquainted. Based on statistical evaluation of the obtained data, it proved that the initial hypothesis claiming the existence of a correlation between driver parameters such as age, gender or length and quality of driver experience and his/her ability to adapt to completely unknown car control systems could be confirmed. The results in this paper may be applied in the cabin and car control system design process, thereby enhancing the user-friendliness of passenger car controls, thereby also indirectly increasing road traffic safety.

Key words: Ergonomics, Experience, Evaluating, Vehicle, Information systems, Safety.

INTRODUCTION

This paper addresses the relationship between a driver's ability to control a passenger car utilizing control systems he or she has hitherto not encountered in the course of his/her driving practice, and driver parameters such as age, gender, or driving experience. The control systems in modern passenger vehicles differ greatly from one another even regarding such fundamental procedures as shifting gears or operating vehicle driver assistance systems (Wang et al., 2007; Bhise, 2012). The impulse to conduct this research was, therefore, an attempt at describing the common situation where an uninformed driver is forced to operate a vehicle that utilizes systems the person has not encountered in course of his/driving practice. This situation directly effects the overall comfort of the driver and thus also impacts the operational safety of the vehicle (Matoušek, 1998; Reed, 1998). Another aspect of this problem is an ever-increasing burden of information drivers of modern automobiles must contend with. With vehicles becoming ever more digitalized and fitted with ever more intelligent information systems, drivers are being subjected to greater and greater challenges regarding the use

of these systems. It is evident that drivers' abilities differ greatly from one another depending on a whole array of parameters which must be further defined. The basis of these differences are disparities in cognitive and social learning abilities, the grounds on which drivers are able to utilize their general knowledge when encountering an unknown situation (Wilson, 1999; Tilley, 2002).

If we define vehicle operation as a work activity, we can assess performance according to parameters that apply to the area of work ability. Work ability is a dynamic system; the personal resources and work environment of a person/driver change throughout his or her life as a result of, for instance, technological advancements or the process of aging. This discovery has resulted in the hypothesis below (Ilmarinen & Tuomi, 2004).

After relevant results were achieved, a hypothesis was determined stating that the ability of a driver to adapt to and operate a vehicle with an unfamiliar control system statistically diminished with increasing age. The ability to adapt is, in this instance, expressed by the time needed to discover and comprehend the principle of use regarding a specific control element (Goudswaard & de Nanteuil, 2000).

MATERIALS AND METHODS

The evaluated data described below was acquired using primary data collection methods in the form of a field experiment and survey. The research was conducted statically in a standing vehicle with primary and information systems activated and at factory settings to ensure identical starting conditions. The tested individuals were positioned in the driver's seat and completed a range of tasks intended to ascertain their ability to orient in an unfamiliar environment.

The Testing Environment

A 2015 Mercedes Benz C220 BlueTec station wagon fitted with maximum interior equipment and furnished with all available information systems was chosen as the test vehicle.

Figure 1. The driver's field of view in the Mercedes Benz C220 test vehicle.

This type and model was selected intentionally because the controls, tell-tales and information systems are very unconventional, and many do not occur in competitors' vehicles of the same category. Thus, a unique test environment was ensured and, subsequently, a greater group of test subjects could be used. Another reason for selecting this vehicle is the fact that components are arranged inside the cabin in an entirely new and revolutionary way, even in the context of the Mercedes brand as a whole. This shift in design is set to be the founding concept for the new cabins of several subsequent series. The location of the gearshift for the automatic transmission, operation of the Command information system, the location of controls on the multifunction steering wheel or the new sunroof controls are among a few of the main innovations.

The Test Group

In order to ensure relevant test results, it was necessary to select a group of subjects that had no prior experience with the test vehicle or any other Mercedes vehicle or model. In this way it was ensured that the tested individual would find him/herself in a truly unfamiliar environment. 150 men and 100 women from 18–70 years of age were selected, from which 121 men and 87 women were included in the study. The results of the other subjects either could not be considered relevant or their testing was impacted by outside influences.

Table 1. The number of tested individuals and relevant parameters

	Number	Average age	Average number of km driven	Average number of vehicles
Men	121*	34	303,885	8.3
Women	87*	33	132,481	6.1
Total	**208***	**33.6**	**232,192**	**7.4**

*Only valid results were included from the original number of 150 men and 100 women.

The Survey and Questions

The research was conducted by asking a number of questions, or, more precisely, by assigning a total of 10 tasks and then timing the test subject in order to ascertain the length of time it takes him/her to complete them. With regard to their importance, tasks were divided into two sets, a primary set of 8 tasks and a control set of 2. Before commencement of each test sequence, the subject was asked about some basic information including whether or not he/she understands the fundamental principles of operating a motor vehicle.

The primary set of tasks, which had been selected to address control and tell-tale elements unique to the test vehicle consisted of the following questions:

1. Activate the wind shield wipers.
2. Turn on the rear window wipers.
3. Open the sunroof.
4. Put the gear lever in the D (drive) position.
5. Turn the central information panel off.
6. Turn of the Head Up Display.
7. Deactivate ESP.
8. Set the navigation system to navigate to a specific address (always the same one).

The control tasks were selected in order to be simple for the tested individual to carry out, thus producing unambiguously different results than the primary tasks. For that reason, tasks were selected to address elements of conventional design that occur both in the test vehicle itself and in practically all other common models of passenger vehicles. The following questions were chosen:

9. Close any air vent.
10. From your current position, open the vehicle's hood.

A time limit of 300 seconds was set for each task. If the test subject was not able to complete a task within the allotted amount of time, the maximum value was recorded along with a note that the task had not been completed. This information was used for further evaluation which is beyond the scope of this paper. The 300 second time limit had been chosen for organizational reasons, but also in light of incidents that occurred during test runs where it was observed that a subject's motivation gradually diminished if he/she was not able to complete a task within a 5 minute interval.

Control tasks were intentionally designed so as not to confirm the above hypotheses and consisted only of activities relating to control elements, the placement of which are generally well known and should be known to the test subjects, regardless of age and experience, or in the case of task 9, are so obvious their completion could be considered trivial. These tasks were expected to produce a **'cannot be confirmed'** result regarding the hypothesis.

RESULTS AND DISCUSSION

Primary task results are listed in Tables 2 and 3. Control task results are listed in Table 4. The regression line, $Y = b1 + b2.ln(X)$, was used as the dependence model. Coefficients b1 and b2 were acquired through linear least squares regression. Logarithmic dependence was selected because it captured the trend better than ordinary linear regression. Logarithmic dependence also reflects the perception of time and other variables.

Each task was assessed against a null hypothesis (b2 = 0) which had been evaluated by an F-test. In this case, the F-test examined whether the model with a b2 coefficient greater than zero expresses the obtained data better than an arithmetic average – in other words, if the dependence model is statistically significant.

For values obtained for both genders, the value of an F statistic was calculated and subsequently compared to the critical value of F distribution of (1, n-2) degrees of freedom at a significance level of 95%. If the value of the F statistic were greater than the critical value, the null hypothesis could be rejected and the model expressing the correlation between age and reaction time in individual tasks could be confirmed. Thus, in addition to the F value in Tables 2 and 3, there is also the 'confirmed' parameter which takes the value of 'yes' or 'no', whereby the value of 'yes' means the hypothesis listed at the start of the paper has been confirmed.

Table 2. Primary Tasks Results for Tasks 1–4

		Task 1		Task 2		Task 3		Task 4	
		F	Confirmed	F	Confirmed	F	Confirmed	F	Confirmed
Sex	**Male** $f_{crit} = 4.01$	50.67	yes	4.25	yes	15.81	yes	25.01	yes
	Female $f_{crit} = 4.24$	22.50	yes	0.48	no	6.81	yes	11.74	yes

Table 3. Primary Tasks Results for Tasks 4–8

		Task 5		Task 6		Task 7		Task 8	
		F	Confirmed	F	Confirmed	F	Confirmed	F	Confirmed
Sex	**Male** $f_{crit} = 4.01$	15.82	yes	14.18	yes	24.37	yes	18.63	yes
	Female $f_{crit} = 4.24$	1.89	no	5.02	yes	5.68	yes	4.56	yes

Table 4. Control Tasks Results

		Task 9		Task 10	
		f_{crit}	Confirmed	f_{stat}	Confirmed
Sex	**Male** F = 4.01	13.3	yes	0.74	no
	Female F = 4.24	0.53	no	0.99	no

Because of the scale of this paper, it is not possible to include all 20 graphs and curves with their individual factors. Therefore, figures below illustrate data for male and female test group in one figure for each task.

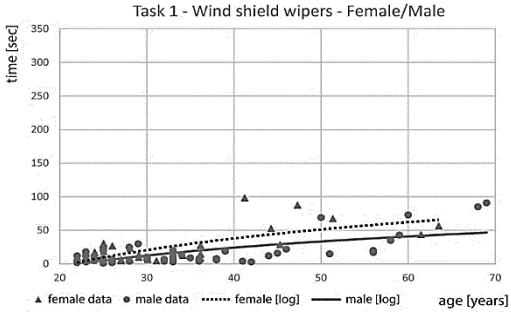

Figure 2. Task 1 – Evaluation of times necessary for test subjects to find the switch for the wind shield wipers. Results measured on a group of men.

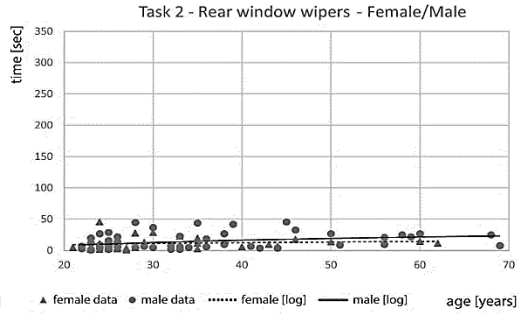

Figure 3. Task 2 – Evaluation of times necessary for test subjects to find the switch for the rear window wipers. Results measured on a group of men.

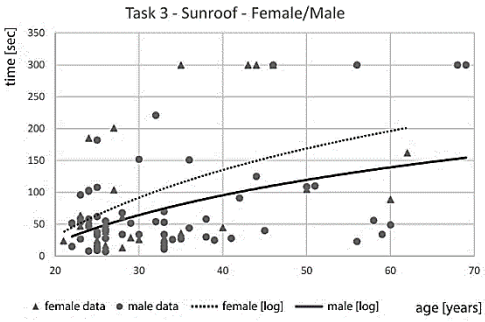

Figure 4. Task 3 – Evaluation of times necessary for test subjects to find the switch for opening the sunroof. Results measured on a group of men.

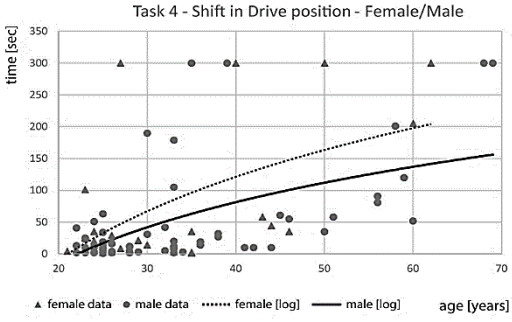

Figure 5. Task 4 – Evaluation of times necessary for test subjects to find the switch gear to be set in to Drive position. Results measured on a group of men.

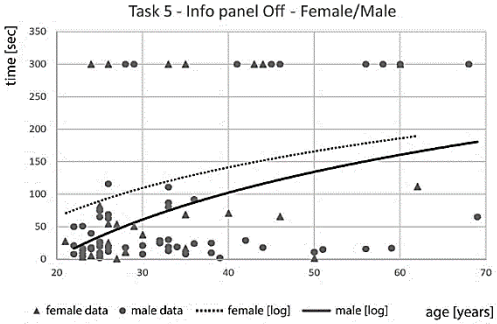

Figure 6. Task 5 – Evaluation of times necessary for test subjects to find the switch for turning off the main info panel. Results measured on a group of men.

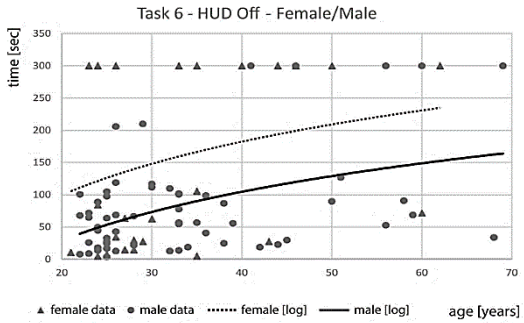

Figure 7. Task 6 – Evaluation of times necessary for test subjects to find how to switch off HUD display. Results measured on a group of woman.

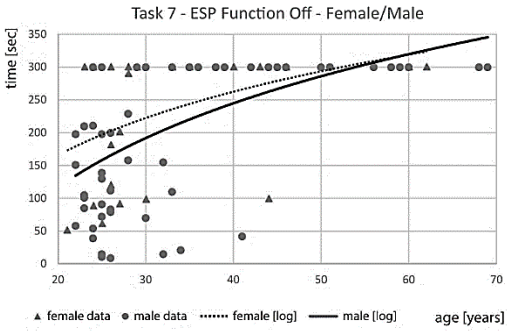

Figure 8. Task 7 – Evaluation of times necessary for test subjects to find how to turn off the ESP function. Results measured on a group of men.

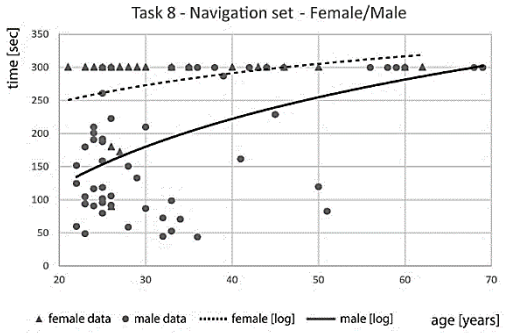

Figure 9. Task 8 – Evaluation of times necessary for test subjects to find how to turn off the ESP function. Results measured on a group of men.

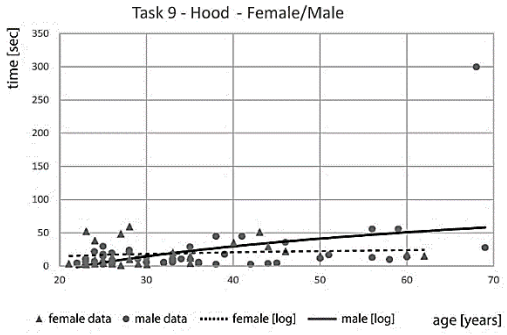

Figure 10. Task 9 – Evaluation of times necessary for test subjects to find the lever to the vehicle's hood. Results measured on a group of woman.

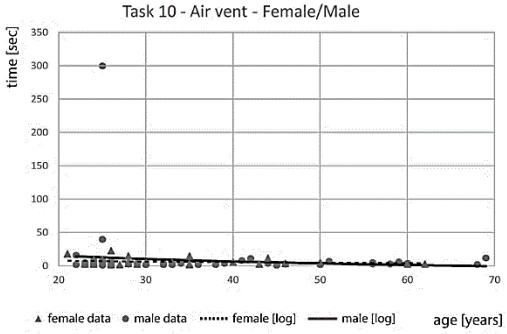

Figure 11. Task 10 – Evaluation of times necessary for test subjects to find how to manage the air ventilation switch. Results measured on a group of man.

In the case of task 2, the hypothesis was not confirmed by the sample group of women, and the sample group of men produced borderline results. This can be interpreted to mean that the controls for front and back wipers were located very near each other, and once the subject discovered how to operate the front windshield wipers, he or she intuitively assumed the controls for the rear wipers would be similar in function and could be found nearby. Many test subjects also accidentally discovered the rear wiper controls while solving task 1. This unfortunately influenced the results of the task. On the other hand, this situation could be construed as exemplifying the principle of learning through the similarity of functions. In the instance of task 5, a correlation was confirmed only among men, the reason being that practically the entire sample group of women was unable to complete the taske regardless of age.

Regarding the control tasks, an evaluation of task 10 involving the all-male group must be put forth. During the assignment where the factor of age was being examined, three men were unable to complete the task due to not being able to physically reach the control, although they new where it was located. If these extreme cases are set aside, the results are practically uniform, regardless of the subject's age.

CONCLUSIONS

On the basis of the foregoing results, it can be stated that the hypothesis voiced in the introduction has been, to a great extent, confirmed. Age has a direct influence on the ability of a driver to adapt to an unfamiliar cabin environment and to new and unknown vehicle control systems. It must be conceded, however, that the time limit given for task completion did bring about some distortion of data, primarily with regard to more complicated assignments. It may be assumed, though, that with regard to this data occurring in the extreme values of the evaluation parameters (advanced age and little experience), increasing the time limit would cause the curve to favour the established hypotheses even more.

The results set forth in this paper could serve as material for further research, helping to refine the above-mentioned findings. The data and hypotheses listed herein could serve as auxiliary factors in automobile design with respect to potential target

groups and customers. For instance, manufacturers of high-class luxury vehicles fitted with extensive, intelligent information systems focus on middle-aged and elderly customers. Unfortunately, elderly drivers are not able to take full advantage of these systems, as was shown by the results herein. That begs the question whether such sophisticated and expensive equipment as these vehicles are furnished with is actually prudent, or whether it has, in extreme cases, a disruptive effect on drivers, lowering operational safety of the vehicle.

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The possibilities of pneumatic reactive stabilization of vehicles

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Abstract. This paper describes a new and original way of car stabilization as an alternative or support for the common ESP stabilization method. It summarizes the properties of present car stabilization systems and their advantages and disadvantages. Then the pneumatic stabilization method is described, which uses compressed air to trigger the necessary reacting forces that are applied to a vehicle in case of the loss of adherence. To prove the new stabilization method, there are stated some basic calculations of the jets and the obtained reacting forces are identified. Finally, the results are discussed and evaluated.

Key words: ABS, ESP, anti-skid systems, pneumatic stabilization, jets, compressed-air reservoir.

INTRODUCTION

Among the basic active and passive safety components, which are included in modern cars, trucks, motorbikes, is the ABS – Anti-Lock Braking System (Zhao et al., 2013; Zhao et al., 2014). This anti-lock system was first introduced in 1978 by firms Bosch and Mercedes-Benz as extra equipment for additional charge at model S and since then it has been widely spread in all models of all car types (Kovanda et al., 2009). Anti-lock braking system is today an obligation without which the car couldn't successfully obtain a homologation.

This article represents the results of our work which is deal with new idea. This was not published till this time. The scope of our work is to explore the possibility of pneumatic stabilization of vehicles entirely by theoretical calculations. The next step of this work was to design the suitable Laval jet and analyze the correction power of pneumatic system according to power of centrifugal force during the car's thoroughfare of curve.

Present state of car stabilization

Together with ABS there are other systems to make comfort better and increase safety such as:

- BAS (brake assistant) – recognizes critical situations and by increasing the pressure it enables the full braking effect.
- ASR – (anti-slip regulation) – prevents slip-spinning at acceleration and moving off by reducing the torque of the engine.
- EMS – reduces the rotary inertia until the powered wheels are at the full adhesion.
- MSR (Motor-Schleppmoment-Regelung) – prevents slip-spinning when braking using the engine.

– ESP (Electronic Stability Program) – prevents the car slipping by braking a selected wheel and thus eliminating the understeering and oversteering.

These systems keep the vehicle in required direction but they have a common disadvantage: there is a limitation of the friction force that can be transferred between the tyres and the surface. This friction force depends for example on the coefficient of friction μ [-], which can sink to the value of 0.1 on ice, compared to values 0.8–0.9 on a dry road. As a result, the transferred force can sink up to 0.1 of the maximal possible value. The corrective forces would be insufficient in such cases to maintain the vehicle on the desired track or even to prevent the vehicle to leave the road.

The weather phenomena plays an important role because there are some unpredictable situations, e.g. when on a very clean road without snow or ice after a machinery clearing, there are some rest icy places – for example on bridges. In these situations the new method of car stabilization could be useful and it could help the driver to solve a problematic situation using additional reactive forces.

A similar solution is using by landing aircrafts, the braking force of the tyres, which is limited due the friction, could be in some situations insufficient to stop the aircrafts within the runway. So another force – the reverse thrust – is applied in such situations that increases the sum of the breaking forces and enables the deceleration more quickly.

Currently used stabilization systems work in connection with the ABS. From the pressure sensor of brake fluid the control unit is informed about the pressure in braking system. The sensors measure the pressure that is created by pressing the brake pedal. Then the ABS control unit compares these signals in both ways. The control unit is informed about the actual pressure in brake system by the pressure sensor of the brake fluid. If interference is necessary, the control unit uses then the actual value of the brake pressure to the calculation of side forces.

The sensor of the longitudinal vehicle acceleration that is the next part of the system is assembled to a car with drive on all wheels. On cars with drive of only one axletree the system calculates the longitudinal vehicle acceleration from the brake pressure sensor signals, from the wheels revolutions sensors and from the information of the engine control unit.

The sensor of the side acceleration of the vehicle informs about side forces applied on the car. This information is important for calculations of the forces that have to be overcome for staying up the car in the intended way. The sensor read stay if the car doesn't revolve around the vertical axis. The micro mechanic system with the double tuning fork from silicon monocrystal placed on the sensor desk is the basic part of the sensor of the rotary speed. The double tuning fork is created by exciting tuning fork and specific tuning fork.

The next part of the car stabilization is the sensor of the steer angle. This sensor sends a partly signal about the steer angle and a partly signal about the speed of the steering-wheel turn. Both signals are at first evaluated in the control unit and they are sent to the control unit of electromechanical servo control (Cerha, 2010). Two absolute magnetic angle sensors are to disposition for the control unit Bosh. These ones (in contrast to incremental sensors) give the information about the steer angle in the full angles range in every time (Kovanda, 2010).

The considerable information part about the car behavior is given by accelerometers. The accelerometers are sensors for measurement of static and dynamic

acceleration. They are used not only for measurement of eccentric or inertial forces, but also for determination of the subject position, it's declination or vibrations, too.

All these car stabilization types use information about the car behavior and about the upon a car acting forces to keeping the car in the intended trajectory by separate wheels braking, by the engine torque changing, or by its redistribution to the separate axletree, respectively on the separate wheel. The common disadvantage of these systems, i.e. the limitation due do friction, has been described above.

MATERIALS AND METHODS

The main scopes of this work are:

1. Make a review and analysis of current posibility of cars stabilization.
2. In theoretical way make a proposal of pneumatic reactive stabilization of vehicles collaborating with ESP.
3. In theoretical way enumerate the parameters of jet and corrections energy at each differencial steps of pneumatic stabilization.
4. Evaulate the possibility of this new reactive stabilization of vehicles.

The new trend during development of adhesive safety system could by the reactive pneumatic stabilization of cars.

This is new original idea using Newton law of action and reaction by the medium of compress air and jets located near the wheels. In critic situations, when the current safety systems are not sufficient, the system blow out air for a short time and according Newton law can help to hold the vehicle in its original direction.

All calculations in this work were made using program Octave – open source alternative to Matlab.

Pneumatic stabilization of car

The new trend in development and system upgrade could be cohesive security system of pneumatic car stabilization based on the Newton's Law of Action and Reaction. Very pressurized air volume will grab in the critical moment form jets placed in car's body corners near from wheels (Direct Industry, 2013; Humidifiers, 2013).

The necessary components are:

- ✓ quick vents at the jets,
- ✓ connection to the ESP control unit,
- ✓ compressed air reservoir,
- ✓ compressed air tubes to the jets,
- ✓ independent jets at wheels.

The compressor will preferentially use electrical energy obtained by car braking or by riding from a hill, or the right moment of the compressor run may be defined by means of GPS navigation data of the car. In this case the energy recuperation can be also used. By this way the ESP control unit can by better prepared to the relevant action and the driver can be informed by a shining pilot light about the impending danger relatively on time. Figs 1 and 2 describe the components´ layout in a car equipped in this way.

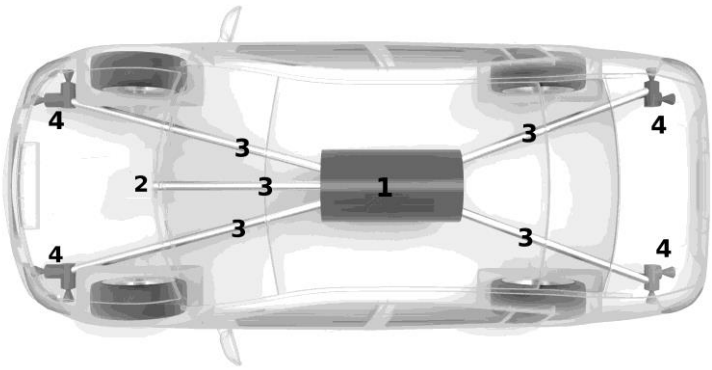

Figure 1. The top view on a car quipped with the pneumatic stabilization system.

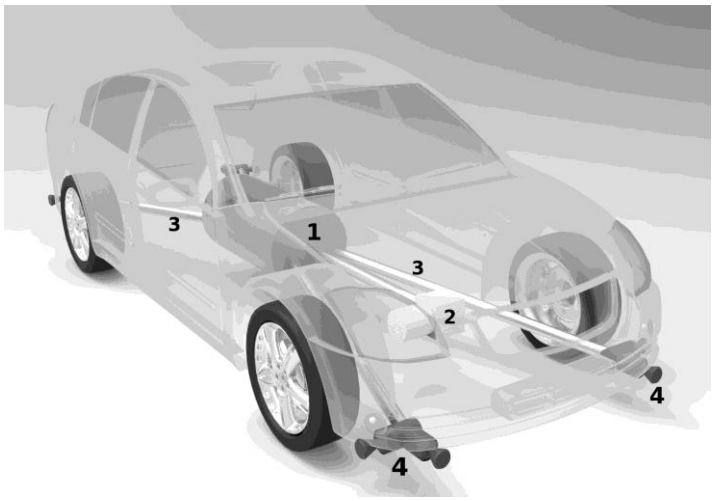

Figure 2. The front view on a car quipped with the pneumatic stabilization system.

In the figures, number **1** marks the compressed-air reservoir, placed in car's center of gravity; number **2** the high-pressure compressor; number **3** labels the compressed-air piping and number **4** the jets in the car's body corners.

A draft calculation of jets

Initial state of the air pressure container:

$$V_{0c},\ p_{0c},\ T_{0c},\ \varrho_{0c}$$

True that:

$$m_0 = V_0 \cdot \frac{p_0}{T_0}\ ; \quad \varrho_0 = \frac{p_0}{r \cdot T_0}$$

where: m_0 [m^3] – the total amount of the compressed air in the pressure container and in the piping (given by the product of the air volume and the air density); V_0 – Initial volume of air at pressure tank; p_0 – Initial air pressure at pressure tank; T_0 – Initial air temperature at pressure tank; ϱ_0 – Initial air density at pressure tank; r – Gas constant (for air 287.04 Jkg^{-1}K^{-1})

$$w_{krit} = \sqrt{2 \cdot c_p \cdot (T_0 - T_{krit})} = \sqrt{\kappa \cdot r \cdot T_{krit}} \quad (1)$$

where: a [m s^{-1}] – Air speed (in this case = $\boldsymbol{w_{krit}}$); w_{krit} – Air speed at critical place of nozzle (narrowest place); T_{krit} – Air temperature at critical place of nozzle (narrowest place); κ – Poisson constant; c_p – 1,004 Jkg^{-1}K^{-1}

$$\dot{m} = m_{krit} = A_{krit} \cdot w_{krit} \cdot \varrho_{krit} \quad (2)$$

Then:

$$a_{krit} = w_{krit} = \sqrt{\kappa \cdot r \cdot T_{krit}} = \sqrt{2 \cdot c_p (T_{0c} - T_{krit})}$$
$$\kappa \cdot r \cdot T_{krit} = 2 \cdot c_p \cdot (T_{0c} - T_{krit})$$
$$\kappa \cdot r = 2 \cdot c_p \left(\frac{T_{0c}}{T_{krit}} - 1\right)$$
$$\frac{T_{0c}}{T_{krit}} = \frac{\kappa \cdot r}{2 \cdot c_p} + 1 = \frac{\kappa \cdot (c_p - c_v) + 2 \cdot \kappa}{2 \cdot c_p} = \frac{\kappa \cdot (\kappa - 1) + 2 \cdot \kappa}{2 \cdot \kappa} = \frac{\kappa - 1 + 2}{2} = \frac{\kappa + 1}{2} = 1.2$$

Further:

$$\varrho_{krit} = \frac{p_{krit}}{r \cdot T_{krit}} \quad (3)$$

$$\frac{T_{0c}}{T_2} = \left(\frac{p_{0c}}{p_2}\right)^{\frac{\kappa-1}{\kappa}} \Rightarrow T_2 = T_{0c} \left(\frac{p_2}{p_{0c}}\right)^{\frac{\kappa-1}{\kappa}}$$
$$\frac{w_2^2}{2} = c_p \cdot (T_{0c} - T_2) = c_p \cdot T_{0c} \left(1 - \frac{T_2}{T_{0c}}\right) \quad (4)$$
$$\frac{w_2^2}{2} = c_p \cdot T_{0c} \left[1 - \frac{p_2}{p_{0c}}^{\frac{\kappa-1}{\kappa}}\right]$$

$$w_2 = \sqrt{2 \cdot c_p \cdot T_{0c} \cdot \left[1 - \left(\frac{p_2}{p_{0c}}\right)^{\frac{\kappa-1}{\kappa}}\right]} = \sqrt{2 \cdot \frac{c_p}{r} \cdot \frac{p_{0c}}{\varrho_{0c}} \cdot \left[1 - \left(\frac{p_2}{p_{0c}}\right)^{\frac{\kappa-1}{\kappa}}\right]} = \quad (5)$$
$$= \sqrt{\frac{2 \,.\, \kappa}{\kappa - 1} \cdot \frac{p_{0c}}{\varrho_{0c}} \cdot \left[1 - \left(\frac{p_2}{p_{0c}}\right)^{\frac{\kappa-1}{\kappa}}\right]}$$

Speed of sound:

$$a = \sqrt{\kappa \cdot r \cdot T}$$
$$a_{krit} = \sqrt{\kappa \cdot r \cdot T_{krit}} \quad (6)$$

The speed of the ejecting air:

$$w_2 = \sqrt{2 \cdot C_p \cdot (T_0 - T_2)} \tag{7}$$

The pressure in the critical place p_{krit} is:

$$p \cdot v^k = const.$$

where: v [m^3 kg^{-1}] – Means specific volume of the air; m_{krit} – Air mass at critical place of nozzle (narrowest place); a_{krit} – Sound speed at critical place of nozzle (narrowest place).

Further considering the Equation of state of ideal gas $p \cdot v = r \cdot T$ we obtain:

$$\frac{p_0}{p_{krit}} = \left(\frac{T_0}{T_{krit}}\right)^{\frac{\kappa}{\kappa-1}} \tag{8}$$

The state in the critical cross-section is given by:

$$T_{krit} = T_{0c} \cdot \frac{2}{\kappa + 1} \tag{9}$$

$$p_{krit} = p_{0c} \cdot \left(\frac{2}{\kappa + 1}\right)^{\frac{\kappa}{\kappa-1}} \tag{10}$$

$$\varrho_{krit} = \frac{1}{V_{krit}} = \frac{p_{krit}}{r \cdot T_{krit}} = \frac{p_{0c}}{r \cdot T_{0c}} \cdot \left(\frac{2}{\kappa + 1}\right)^{\frac{1}{\kappa-1}} \tag{11}$$

The amount of air flown through the jet:

$$\dot{m} = A_{krit} \cdot \sqrt{\kappa \cdot r \cdot T_{krit}} \cdot \varrho_{krit} = A_{krit} \cdot \sqrt{\kappa \cdot r \cdot \frac{\kappa}{\kappa - 1} \cdot T_{0c}} \cdot \varrho_{krit} \tag{12}$$

where: $\dot{m}$ [kg s^{-1}] – Mass flow rate; A [m^2] = Cross-section area of the jet, A_{krit} = Nozzle aperture at critical place of nozzle (narrowest place); ϱ_{krit} = Air density at critical place of nozzle (narrowest place).
For further calculations for a given time we consider: $t = t_0 + \Delta t$

After application of the adiabatic equation we obtain:

$$p_0 \cdot {V_{0c}}^{\kappa} = (p_{0c} - \Delta p) \cdot (V_{0c} + \Delta V)^{\kappa} \tag{13}$$

For further calculations we choose:

$\Delta V = 0.01\ m^3$

1. Step $t = t_0$, $V = V_0$, $p = p_0$
2. Step $t + \Delta t$, $V = V_0 + \Delta V$, $p = p_0 - \Delta p$

Given by the equation of state: $\Delta m = \Delta V \cdot \varrho_0 = \Delta t \cdot \dot{m}_{krit} \Rightarrow \Delta t \frac{\Delta m}{\dot{m}_{krit}}$.
In the following step, we repeat the calculations with new values:

$t_{i+1} = t_i + \Delta t_{i+1}$
$T_{i+1} = T_i - \Delta T_{i+1}$
$t_{i+2} = t_{i+1} + \Delta t_{i+2}$
$p_{i+2} = p_{i+1} - \Delta p_{i+2}$
$T_{i+2} = T_{i+1} - \Delta T_{i+2}$

The outflow speed from the jet w_2 is a speed, by which during the expansion of the air in the cross-section A_2 the pressure decreases to the atmospheric pressure $p_2 = p_e$. From the Continuity equation: $\dot{m} = \dot{m_{krit}} = A \cdot w \cdot \varrho = A_2 \cdot w_2 \cdot \varrho_2$

Using: $T_2 = T_0 \cdot \left(\frac{p_e}{p_{0c}}\right)^{\frac{\kappa-1}{\kappa}}$ $\varrho_2 = \frac{p_2}{r \cdot T_2}$ $w_2 = \sqrt{2 \cdot c_p \cdot (T_0 - T_2)}$

The output cross-section of the jet will be: A_2

$$\dot{m}_{krit} = A_2 \cdot \varrho_2 \cdot w_2 \Rightarrow A_2 = \frac{\dot{m_{krit}}}{\varrho_2 \cdot w_2} \tag{14}$$

The resulting corrective force of the ejecting compressed air in the first stage of expansion (an under-expanded jet):

$$F = \dot{m}\, w_2 + A_2\, (p_2 - p_e) \tag{15}$$

where: F [N] – The obtained corrective force.

In case of an ideal expansion is true:

$$F = \dot{m} \cdot w_2 \tag{16}$$

In case of an over-expanded jet is true:

$$F = \dot{m} \cdot w'_2 \tag{17}$$

where: w'_2 [m s^{-1}] – The imaginary speed during the ideal expansion to the pressure p_e.

The control calculations were implemented for the jet with the output diameter 35 mm, in the narrowest diameter 15 mm, pressure values in the air reservoir of 10 MPa, 15.5 MPa and 20 MPa, volume of the reservoir 0.05 m^3, car mass 1,000 kg, car speed 80 km h^{-1} (22.2 m s^{-1}) and the radius of turn 50 m. Fig. 2 shows the results of the calculations for the given initial pressure values.

The final air pressure in the reservoir decreased to the value of 3.5 MPa within 3.2 s. Considering the initial temperature 323 K, the sum of acting forces reaches the value 3.8 kN for the initial air pressure value of 10 MPa, 6.3 kN for 15.5 MPa and 8.3 kN for 20 MPa. The centrifugal force in the curve reaches the value of approximately 10 kN for the defined car and conditions. The results for selected initial pressure and temperature values are represented graphically in Figs 3, 4, 5.

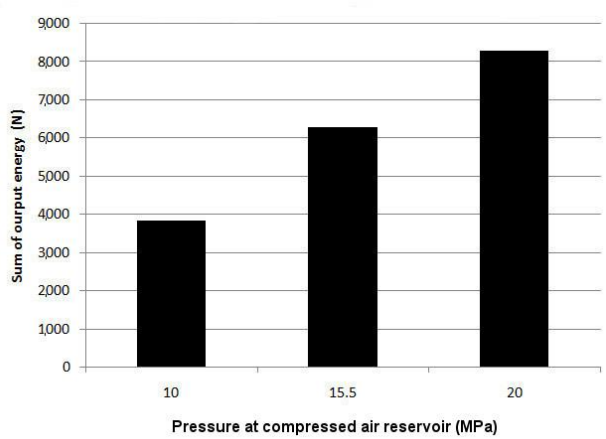

Figure 3. Dependence of the sum of the reacting forces on the initial air pressure for 323 K.

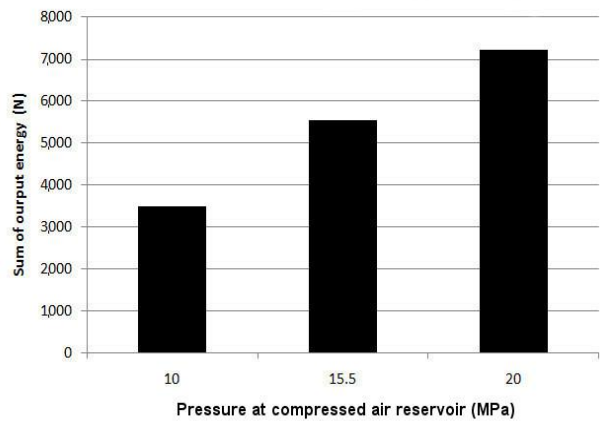

Figure 4. Dependence of the sum of the reacting forces on the initial air pressure for 423 K.

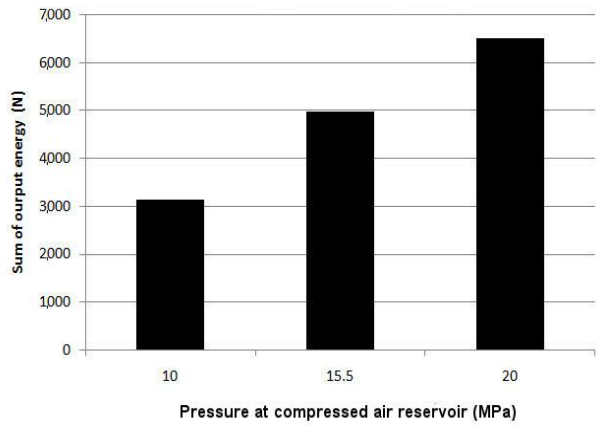

Figure 5. Dependence of the sum of the reacting forces on the initial air pressure for 523 K.

The calculated sum of reacting forces reaches the value up to 8.3 kN, which is about 83% of the centrifugal force acting on the vehicle. The value increases with increasing initial pressure in the container, and decreases with increasing temperature. Thus, cooling of the compressed air is necessary for better efficiency of the system.

However, we must consider, that the total reaction force acting on the vehicle is a sum of the considered pneumatic reaction forces and the forces given by the conventional stabilization system, i.e. the ESP that are nevertheless strongly dependent on the surface conditions of the road. In some cases the adhesive stabilization system may be sufficient, in other cases the pneumatic stabilization system may act as a safety margin to the conventional systems. With the highest initial pressure, we can virtually obtain the required corrective force without any further adhesive forces.

CONCLUSION AND DISSCUSION

We introduced a new and original method, the comparable studies were not published yet. The pressurized air is used to compensate the forces acting on a vehicle. We have proved that the described system may considerably help the present adhesive-based stabilization systems like the ESP. The control calculations demonstrated the possibility to compensate the centrifugal force acting on the given vehicle in the ranges of 38% to 83%, which can considerably help the adhesive forces or even virtually to compensate them to stay the car in the intended trajectory.

The primary goal of the this work was to explore the possibility of using this idea only by help of mathematics calculations (see appendix) an to design the suitable Laval jet. This is only the purely theoretical work with original and promising results. At real this is an upgrade of current ESP system in case of adhesive stabilization is not fully sufficient and there is the risk of emergency situation. We do not suppose to use this at everyday usage.

The next step depends on manufacturers if they decide to use the system as a way to make the car operation better and safer. Add-on systems could make cars more complicated and more expensive. But the massive production can lower the costs. The price of the life is incalculable. As for the space needed for this technical device, it may not be a big problem because the device can be placed at the bottom of the car and it will not occupy more space than for example the gas reservoir that is used in cars equipped with this invention.

The pressure at pressure tank is according today's material a technology potential. At industries there are using pressures around 30 MPa and more normally. This study is at the begging so the financial costs are higher then usually but this is normal at every development. I'd like to refer the costs of ABS of beginning its development. And today it is the basic of safety cars. Influence to environment is always small since this technology use the clear pressured air. And also in this case we can omit the effect of speed and direction of wind because of the speed of air from jet is multiply bigger. The scope of this study was only to confirm the physical possibilities of my idea and to design the suitable Laval jet. The statistics methods were not used because the calculation using math program gives us relevant results.

The technology of the adhesive solutions is still limited due to the friction between the tire and the surface, but the pneumatic technology may still develop further, producing higher pressures and thus higher compensation forces. Considering this limitation of the adhesive-based systems, this could be a revolutional solution for the future.

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Basic theory and methods for managing energy efficiency in consumer systems

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Abstract. In this article, we present a scientifically proven methodology for monitoring and controlling of industrial energy efficiency in consumer power systems (CPS) – an original method of finite relations (MFR), which uses relative energy intensity resulting from energy use in the technology of the enterprise as the main indicator of innovative energy efficiency. The differentiation algorithm and control of energy consumption are based on the effectiveness of energy use in power technology processes which ensure delivery of technologically expected results. The main provisions of the method are confirmed by experiments and tested under production conditions. The research results correspond to the basic principles of the global energy efficiency practices (integrated approach to the design and the principle of sustainable development), but yielded a number of more specific solutions.

Key words: energy saving, power consumption of production, criteria of energy efficiency, consumer power system.

INTRODUCTION

The quest for energy conservation by individual countries and on a global scale, due to a variety of obvious reasons, has a fairly long history, and the analysis of this quest allows you to make some conclusions. In particular, in production, which is almost the revealing (Stasinopoulos et al., 2012) point of the prospect of energy consumers' systematic approach to the design and engineering of sustainable development. Such recommendations are convincing and effective because of a large number of separate illustrated practical examples. However, their implementation requires in each case integrated energy to generalize the method of calculation, control and monitoring performance, providing energy-saving properties of the complex. This goal is the subject of this article. It should also be borne in mind that energy efficiency is closely related to the competitiveness of production enterprises, thus, to improve their economic situation. To find answers to the question 'Why the real engineering as a product of the exact sciences does not offer appropriate solutions for energy savings?' it is important to determine the prospects for the development of energy conservation. One possible answer lies in the fact that engineering knowledge is created mainly by studying the device of power equipment and its performance for a particular use. With such knowledge, the energy (Q) (the main carrier of action) and power (P) (indicator mode

motion energy) are significantly fragmented, as their practical connection with total consumption is a much more complex mathematical operation of integration of functions, which is not always feasible.

MATERIALS AND METHODS

Cash-measuring finite ratio method (MFR) is based (Karpov & Yuldashev, 2010) on the ability to measure power at any function of power and allows circumventing this difficulty with the in-depth knowledge of experts. The ratio of energy input to a technical element (Q_s) and the output energy of the element (Q_e) forms the performance indicator (Q_E) of the energy passing through the element.

$$Q_E = \frac{Q_s}{Q_e} \quad (1)$$

Versatility and availability of the values of this indicator made it possible to obtain a numerical estimate of the energy of not only individual technical elements and engineering systems.

From the standpoint of energy efficiency, it is most relevant to consider energy companies and products acceding the market competition. Expediency is not explained by the fact that the definition of the energy intensity of production is not available, and that the ability to manage power consumption at present is intuitive and heuristic even for experts, and not provided with a special methodology. The task of energy product management (minimization) led to the solution for the energy companies (Karpov et al., 2014) as energetic consumption systems (ECS), creating a well-defined structure of the energy intensity of production and the method of finite relations, and allowing us to analyse the structure and justify optimization of management solutions. Functional diagram of the ECS generally shown in Fig. 1.

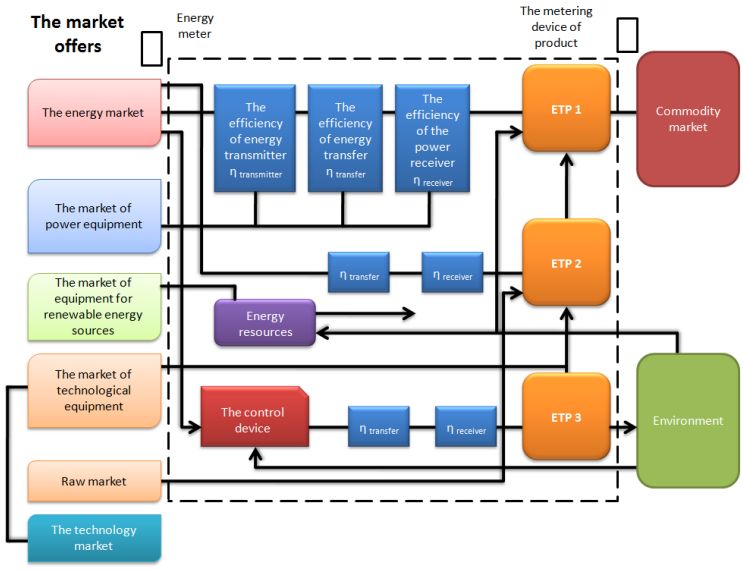


Figure 1. Block diagram of the ECS.

The generality of the scheme for the energy of different companies is reflected by the presence and the ability to measure all kinds of energy input, the presence of power lines supplying power directly to the receivers, as well as by specification of the bilateral market restrictions in the form of market quotations on the left side (with the payment of expenses) and the consumer market for the implementation of the Right product (with the refund in the form of income) on the right side. Basically, new circuit elements are power technology processes (ETP). The diagram shows three kinds of ETP, reflecting all of their real-life variety. Each ETP is accompanied by information about what type of energy is used, which object is the energy impact, a result of figure *R* to be obtained on production technology. Of particular importance in the scheme of introducing the ETP of ECS is the fact that they allow to calculate the minimum energy Q_{min} consumption for processing the results of *R*, which makes the formulation of the problem to optimize the energy efficiency more correct.

$$Q_{R\,i}^{spec} = Q_i^{spec} \cdot R_i \tag{2}$$

$$Q_{min} = \sum_{1}^{n} Q_{R\,i}^{spec} \tag{3}$$

where Q_i^{spec} – grounded theoretical energy density is determined in general physical constants, regulatory or experimental data.

In addition, calculations for higher ETP require the knowledge of experts – power engineering on the physical laws of the impact of energy on different media. According to a scientific school that developed at the emergence of the theory of energy saving, AEDs contain an empirical examination of the ETP, which aims to determine the effect of experimental energy losses on energy consumption results of ETP. The estimated value of energy losses on each item and each energy line of ECS is determine from and justified based on the values of energy consumption and according to the structure of the energy intensity of production, taking into account the financial costs and urgent, medium- and long-term measures to reduce the energy intensity of production.

The methods developed make it possible to calculate the impact of energy savings in the economic performance of the company (Kabanen & Karpov, 2014). Our studies have shown that the practical use of this feature provides a basis for the transfer of activities of energy companies only in the professional section of effective energy management; energy efficiency increases because of the yield of the company. It is also established that, presumably, involved commercialized energy gets its own economic indicator - the private rate of return (α_E):

$$\alpha_E = \frac{P_p}{\mathrm{C_T} \cdot Q_{el}} = \frac{Q_{el}^{market}}{Q_{el}} \tag{4}$$

where P_p – price of products; $\mathrm{C_T}$ – energy tariff; Q_{el} – power consumption of enterprise; Q_{el}^{market} – energy intensity of production of a market.

The positive effect of energy savings on the economic condition of the company strengthens the confidence in success and solves the problem of sustainable

development, which is largely constrained to the high cost of elimination of environmental damage.

In theory formed the basis of energy-saving consumer energy systems put the main, but not deeply studied the theorem of calculus (Fermat's , Rolle's, Lagrange's, Cauchy's), numerical integration of functions of one variable, the average power is determined by the energy value or the increment. Largely, the provisions of the new applications of the theory of energy saving may be differential inequalities in integral calculus.

RESULTS AND DISCUSSION

To illustrate the validity of the use of the theory described in a practical energy saving data, we provide an empirical examination of a single electronic trading platform – water heating in electric heater. According to the classification adopted in the framework of the ECS, heating process can be attributed to the ETP2 (sub-processes). In this process, electric energy is converted into heat by means of a tubular electric heater (heater), which then has an impact on the volume of the energy of the heated fluid to raise its temperature to a value predetermined by the technology.

The object of examination is a water heater type EVBO-20 / 1.25, intended for heating water. The main parameters of the heater: the volume of heated water is 20 litres; rated power heater – P_s = 1.25 kW.

Based on the passport data of the heater, we selected baseline conditions for studies – the initial water temperature $T_{initial}$ = 20 °C and the required heating temperature T_{final} = 60 °C.

Preliminary analysis of the ETP test allowed to determine the theoretical amount of energy required to produce a given technological outcome (increase in temperature from the initial to the final), by the transition from the general formula (2) to the private characteristic of the process:

$$Q_R^{spec} = c \cdot m \cdot \left(T_{final} - T_{initial}\right) = Q^{spec} \cdot R\,, \qquad (5)$$

where m – mass of water; kg; c – the specific heat of water kJ (kg °C)$^{-1}$.

In this example, Q^{spec} is determined based on the physical quantities in the calculation constant. For the test, a theoretical heating energy consumption was $Q_R^{spec} =$ 3,352 kJ.

Theoretical time to achieve results t^{spec} can be determined by a calculation using the formula:

$$t^{spec} = \frac{Q_R^{spec}}{P_s}; \qquad (6)$$

if t^{spec} = 2,682 c.

An empirical assessment of the impact of energy loss results – ETP is based on determining the relative power consumption of the process (Q_E). For this indicator, we carried out, according to MFR, simultaneous measuring and recording of the amount of electrical energy consumption (Q_s) and temperature (T), which characterizes the integral impact of energy on the environment:

$$Q_E = \frac{Q_s}{Q_R^{spec}} = \frac{\int P_\varphi(t)dt}{Q^{spec} \cdot \left(T_{final} - T_{initial}\right)}. \quad (7)$$

Maximum efficiency of the process is characterized by a lack of energy loss (ΔQ = 0), in this case, all consumed energy is spent to achieve the result of the process ($Q_s = Q_R^{spec}$) and $Q_E = 1$ if ΔQ = 0.

The controlled experimental studies of the process implemented in the course of the examination allowed to determine the loss of energy (ΔQ) and assess the impact of this on the relative energy content (Q_E) with appropriate operational and regulatory constraints:

Experiment №1. The definition of 'congenital' energy efficiency of equipment due to the level of engineering in the design phase (U = 220 V, $T_{ambient}$ = 22 °C);

Experiment №2. Investigation of the effect of ambient temperature from the decrease of energy in equipment (U = 220 V, $T_{ambient}$ =1 °C). The selected ambient temperature, according to the passport data of the test heater, is the minimum acceptable for its operation;

Experiment № 3. Investigation of the impact of reducing the voltage on the energy loss (U = 198 V, $T_{ambient}$ = 22 °C). The values of the steady voltage deviation in accordance with GOST 32144-2013 should not exceed ± 10% of nominal;

Based on the performance indicators derived from the research, we developed measures for managing energy efficiency in the process – the application of thermal insulation to reduce heat loss through the surface of the tank heater.

Experiment № 4. Analysis of the application of thermal insulation to reduce heat loss through the surface of the tank heater. (U = 220 V, $T_{ambient}$ = 22 °C). For this, we chose insulation with a thermal conductivity λ = 0.038 W (m °C)$^{-1}$.

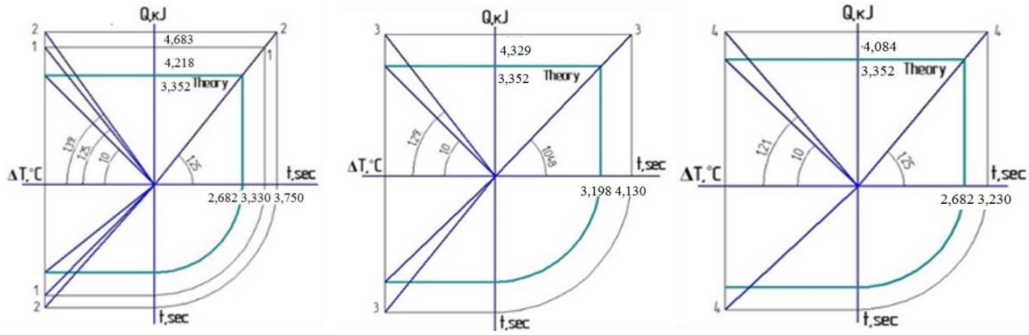
The results of experimental studies allow speaking with confidence about the universality of the finite relations, as well as its applicability in practical energy saving at all stages of the life cycle of the ECS.

Table 1. Main results of experiments

Experiment №	U, V	$Q_н$, kJ	$Q_к$, kJ	ΔQ, kJ	t, sec	$P_φ$, kW	$Q_э$
1 ($T_{ambient}$ = 22 °C)	220	4,218	3,352	866	3,330	1,248	1,258
2 ($T_{ambient}$ = 1 °C)	220	4,687	3,352	1,335	3,750	1,251	1,398
3 (U = 198V)	198	4,329	3,352	977	4,130	1,048	1,292
4 (thermal insulation)	220	4,084	3,352	732	3,230	1,254	1,218

The total number of sources for further analysis of the process variables is three (electricity consumption Q_s, the result of ΔT, and the process t), so their values are conveniently displayed in the form of points in a plane coordinate system, forming the original idea, which is proposed to call the universal energy diagram (Fig. 2).

On one chart, few circuits may be delayed, illustrating the various experimental conditions, however, the comparison will always be carried out with a theoretical outline, reflecting the highest energy efficiency (see Fig. 2, the circuit 'Theory').



a) b) c)

Figure 2. Energy diagram of the heating process: a) experiments №1 and №2, b) experiment №3, b) experiment №4.

Analysis of the results of empirical examination revealed common patterns of change in the efficiency of heating due to the effect of energy loss, and to carry out verification, we transformed them into the form of a numerical estimate of the relative energy consumption and energy saving measures.

The results of experimental studies allow speaking with confidence about the applicability of the method of finite relations in practical energy saving, as well as its universality. Subject to all major trading and enterprise information-measuring systems that implement the proposed method, it is possible to create a global system for monitoring energy efficiency in terms of the relative energy consumption. Such a system will ensure continuous monitoring of the use of resources to determine the optimal strategy for managing power consumption of products. Thus, experts of energy service businesses are able to affect the stability of the technical and economic spheres of production by means of operational performance.

A similar examination was carried out to change the electrical energy into mechanical energy – induction motor (IM).

To control the energy efficiency of IM, according to MFR, you must conduct periodic systematic measurement of relative energy intensity of his work during the whole period of operation.

In reference books and catalogues of manufacturers, the value of the energy characteristics of induction motors (IM) (η and $cos\varphi$) are given at partial load (25; 50; 75; 100 and 125% of rated power). However, in the real world, there is a difference in the energy performance of new blood pressure on the passport, which can be evaluated numerically in terms of the relative energy consumption.

The main characteristics of the studied blood pressure passport are type-AO2-51-4S2; Power – 7.5 kW.

For experimental studies to determine the relative power consumption of AD and DC motors, we developed a universal test bench, equipped with an electromagnetic brake that simulates the load on the motor shaft. For the measurement, recording and archiving of operating parameters of the IM, the stand is equipped with information-measuring system (IMS), which allows you to receive energy efficiency index of algorithms CIE (Table 2).

Table 2. Relative energy intensity of the work of IM stated in the passport and the results of experimental studies

Load factor of IM	Relative energy intensity IM, Q_E	
	Q_E^{namepl}	Q_E^{exp}
0.25	2.1	2.77
0.50	1.46	1.77
0.75	1.33	1.50
1.00	**1.29**	**1.44**
1.25	1.31	1.48

The results of experimental studies demonstrate the applicability of the method of finite relations to determine the energy efficiency of IM. Analysis of the results of calculations and experimental data leads to the conclusion that the blood pressure has a minimum relative power consumption at a rated load of operation $Q_E^{pasport} = 1.29$ and $Q_E^{exp} = 1.44$. By reducing the load factor below 0.75, there is a significant deterioration of the energy characteristics and an increase in relative energy intensity of the work of IM.

CONCLUSIONS

Using this method in practical energy saving will enable to assess the state of electric energy and to develop measures to manage energy efficiency, using known methods and means (regulation, control modes, etc.). Or replace them with new ones with improved power parameters. Thus, experts of energy service businesses are able to affect the stability of the technical and economic spheres of production by means of operational performance.

The development presented in the article should ensure the acceleration of the progressive development of energy consumers. As a rule, producers have one significant unconventional energy resource (energetic discharges, local wind energy, solar radiation, water flows, available biomass energy). The effectiveness of the use of this resource will be greatly enhanced by the reduction in the state of power of the consumer system.

It should also provide a solution to energy problems by using specially trained personnel. The methods outlined in this article can be used to develop educational programs of multi-level education, which provide consistent knowledge to the audience about the controlled system.

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Monitoring oil degradation during operating tests

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Abstract. This paper deals with a lifetime test of the hydraulic and transmission oil, Shell Spirax S4 ATF (Universal Tractor Transmission Oil (UTTO)). This fluid was used in the hydraulic and transmission circuit of a John Deere 5720 tractor. The fluid was assessed in terms of a possible contamination of chemical elements. Fluid samples were taken from a John Deere 5720 tractor at intervals of 250 engine hours. These samples were subjected to an IR spectroscopy analysis, a measuring of the kinematic viscosity at 40 °C, and differential scanning calorimetry (DSC). The spectrometer, Spectroil Q100, has been used to monitor chemical elements.

Key words: hydraulic and transmission circuit, tractor, chemical elements.

INTRODUCTION

Hydraulic equipment is widely used in powerful mechanisms that are part of agricultural and forest machines, as well as in many other areas. The development of modern hydraulic components is aimed at increasing the transmitted power, reducing energy intensity (by the use of smaller reservoirs of hydraulic fluid), minimising environmental pollution, and increasing technical life and machine reliability (Tkáč et al., 2008; Hoffmann et al., 2013). Hydraulic and transmission fluid requires quality parameters to be monitored (regarding any concentration of metallic elements and any concentration of chemical elements representing the additives). Fluid purity is one of the most important features in the entire process (Máchal et al., 2013; Majdan et al., 2013). Very often, the questionable purity and general technical condition of hydraulic and transmission fluid are frequent the causes of failures in transmissions or hydraulic system in tractors. Contaminated fluid creates a risk to the machine in terms of wear and failure (Tulik et al., 2013). Pollution (a concentration of metallic elements) is dangerous because it accelerates the degradation and oxidation processes in the fluid. If the fluid is contaminated by dirt above the permitted level, it must be replaced (Majdan et al., 2014).

Universal Tractor Transmission Oils (UTTOs) are designed for hydraulic and transmission systems in agricultural tractors. These fluids provide lubrication functions for the gear box and the transmission of energy in the tractor's hydraulic system (Hujo et al., 2013). The friction points in the hydraulic and transmission circuit are made from several metals (mostly iron, aluminium, and copper components) (Kumbár et al., 2014). For this reason there is a need to check for other metals, such as aluminium, copper, chromium, lead, tin, nickel, silver, etc (Kumbár & Dostál, 2013). The behaviour of the oils at low temperatures is also important. Bai & Bai (2014) wrote that, in many cases, thermal analysis is required, especially for fluids that are viscous and have high freezing points or in cold weather conditions. Schubring (2009) deals with problems related to freezing of oil. Differential scanning calorimetry (DSC) has been used successfully in oil investigations for quite a long period of time (Kök et al., 1999; Santos et al., 2005). DSC has some advantages over other classic detection methods, as it is rapid and does not require excess sample preparation or solvent utilisation and, therefore, it is an environmental friendly technique (Dahimi et al., 2014).

MATERIALS AND METHODS

An operational test of a specific hydraulic and transmission fluid, Shell Spirax S4 ATF, was set at 500 engine hours (EH). Oil samples were taken after completing 250 and 500 engine hours. Subsequently, fluid samples were collected for analysis and the detection of any contamination. As regards the utilisation of hydraulic and transmission fluid in a machine, the most important factor is knowing the running properties of the fluid, ie. knowing the effect of the fluid on the technical condition of hydraulic and transmission system parts.

The hydraulic and transmission fluid, Shell Spirax S4 ATF, was used in the hydraulic and transmission circuit of a John Deere 5720 tractor. Table 1 shows the basic technical parameters of the hydraulic and transmission fluid.

Table 1. Technical parameters for Shell Spirax S4 ATF

Properties	Unit	Amount
Kinematic viscosity at 40 °C	mm s^{-2}	33.2
Kinematic viscosity at 100 °C	mm s^{-2}	7.2
Density at 15 °C	kg m^{-3}	847.0
Flash point	°C	185.0
Pour point	°C	-48.0

Determining the chemical composition of hydraulic and transmission fluid is something that has been measured using Spectroil Q100, which is a completely solid state spectrometer. Using this spectrometer, measurements can be taken of trace levels of elements which have been dissolved or deposited as fine particles in mineral or synthetic oil-based fluids using a long-established and reliable technique that involves a rotating disk electrode. This spectrometer meets the requirements of the ASTM D6595 standard when it comes to determining the wear rate of metals and the levels of contaminants in lubricating oils and hydraulic mixtures that are being used (Kumbár et al., 2014). The following parameters were evaluated:

- ✓ any concentration of metallic elements (Ag, Al, Cu, Cr, Fe, Mg, Mo, Mn, Ni, Ti, Si),
- ✓ any concentration of chemical elements representing the additives (B, Ca, P, Zn),
- ✓ kinematic viscosity at 40 °C,
- ✓ the pour point by means of differential scanning calorimetry.

Differential scanning calorimetry (DSC) is a technique in which any difference in heat flow (power) to a sample and to a reference is monitored against time or temperature while the temperature of the sample, in a specified atmosphere, is programmed (Haines, 1995). The differential method compares the thermal behaviour of reference material against a sample. This method provides information on thermal effects which are characterised by an enthalpy change and by a temperature range, such as phase transitions (melting, crystallisation, etc).

Differential scanning calorimetry (DSC) was carried out on a Mettler Toledo DSC unit (Fig 1). Samples with a weight of between 8–13 mg were hermetically sealed in aluminium crucibles and thermally treated at a speed of heating of 2K min^{-1} within a temperature range of 20 °C and minus 60 °C. The measurement was carried out in an ordinary air atmosphere. As a result we got a DSC thermogram, which was evaluated using STAR^e software.

Figure 1. Mettler Toledo DSC unit.

RESULTS AND DISCUSSION

Table 2 show an increase in the concentration of chemical elements in hydraulic and transmission fluid during tractor operation. A concentration of chemical elements represents fluid contamination. Any increase of chemical content (for 250 engine hours) and then decrease of chemical content (for 500 engine hours) is caused by the filtration of particles in the tractor filter system. Hydraulic fluid (fluid filling) is not homogeneous. During the tractor being operated under normal conditions, wear particles are absorbed by the tractor's oil filter system.

Table 3 shows the base elements that characterise the set of additional packages. The chemical properties of the hydraulic fluid, being used as the quality evaluation parameters, were monitored in publications by authors Kučera & Rousek (2003), Majdan

et al. (2014), Phillips & Staniewski (2016). The authors focused on kinematic viscosity, additive content, and fluid contamination.

Table 2. Concentration (mg kg^{-1}) of chemical elements

Chemical content	Count of engine hours		
	0	250	500
Ag	0.04	0.09	0.1
Al	0.39	3.41	2.87
Cu	0.29	20.47	19.13
Cr	0	0.64	0.79
Fe	0	49.63	37.24
Mg	11.73	12.85	11.5
Mo	0.48	1.47	1.85
Mn	2.8	4.17	3.98
Ni	0.55	0.62	0.69
Ti	1.2	1.19	1.7
Si	4.44	14.7	12.16

Table 3. Concentration (mg kg^{-1}) of chemical elements representing the additives

Chemical content	Count of engine hours			ΔE (%)
	0	250	500	
B	83.34	69.28	63	24.41
Ca	2531	2,196	2,067	18.33
P	1,222	1,195	1,153	5.65
Zn	1,438	1,242	1,230	14.46

The biggest increase of deposited metals in the oil were observed in relation to copper (Cu), iron (Fe), and Silicon (Si). Iron (Fe) and Silicon (Si) are used as construction material in the transmission, and copper (Cu) is used as construction material in the oil cooling system. Any concentration of Cu, Fe, and Si are standard values of content, as shown in publications by the authors, Kumbár et al. (2014) and Tarasov, et al. (2002). Other changes in the chemical content of hydraulic and transmission oil are almost negligible.

The concentration levels of any additives were monitored on the basis of the relevant content of chemical elements (B, Ca, P, and Zn). The concentration of these chemical elements decreases due to a gradual depletion of additives in the hydraulic and transmission fluid. A decrease in the concentration of chemical elements that represents additives, ΔE, was calculated according to Eq. (1). A decrease in the content of these chemical elements is calculated by using the following formula:

$$\Delta E = \frac{E_0 - E_{500}}{E_0} \cdot 100 \qquad (1)$$

where: ΔE is an increase of the content of chemical elements (%); E_0 is the content of chemical elements at zero engine hours (at the start of the test) (mg kg^{-1}); and E_{500} is the content of chemical elements at 500 engine hours (at the end of the test) (mg kg^{-1}).

The largest decrease was observed in the measuring of boron (B) at 24.41%. Zinc (Zn) is used as an anti-wear agent or as an antioxidant. Hydraulic and transmission oils with zinc additives that are too high have the habit of leading to metal corrosion as they chemically attack the metal surfaces (Nicholls et al., 2005). The decrease was observed in the measuring of zinc (Zn) at 14.46%. This decrease is relatively low according to publications by the authors Kumbár et al. (2014) and Hernández Battez et al. (2008).

Table 4 gives acceptable accuracy readings for chemical elements as a function of the standard concentration. Column 1 in Table 4 gives concentration values in mg kg^{-1}. For example, if a 50 mg kg^{-1} multi-element standard is burned on the Spectroil Q100, an average of ten burns for iron is expected to be 50 mg kg^{-1}, plus or minus 8.29 mg kg^{-1}. Therefore, an average in the range of 41.71 to 58.29 mg kg^{-1} would be acceptable.

Table 4. Acceptable accuracy indices for chemical elements (mg kg^{-1}) (Spectroil M Family of Spectrometers, 2009)

Conc.	Al, Cr, Ni, Si	Ti, B	Fe, Ag, Mo	Cu, Mg	Pb, Sn	Zn	Na	V, Mn, Cd, Ba	Ca	P
0	0.88	0.89	0.91	0.92	1.60	0.96	1.01	0.50	0.50	N/A
5	1.20	1.30	1.50	1.61	1.98	1.99	2.59	N/A	N/A	N/A
10	1.59	1.78	2.21	2.44	2.43	3.19	4.36	1.50	1.50	N/A
30	3.33	3.93	5.23	5.91	4.47	8.15	11.6	3.25	3.25	15.5
50	5.12	6.14	8.29	9.43	6.64	13.1	18.9	5.50	5.50	18.2
100	9.65	11.7	16.0	18.2	12.2	25.6	37.1	10.0	10.0	20.0
300	27.8	33.9	46.7	53.5	34.3	75.6	110	32.0	32.0	35.0
500	46.0	56.1	77.5	88.8	56.6	126	183	53.0	53.0	60.0
700	64.2	78.3	108	124	78.8	176	255	N/A	N/A	N/A
900	82.4	101	139	159	101	226	328	95.0	95.0	105
5000	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	500	500

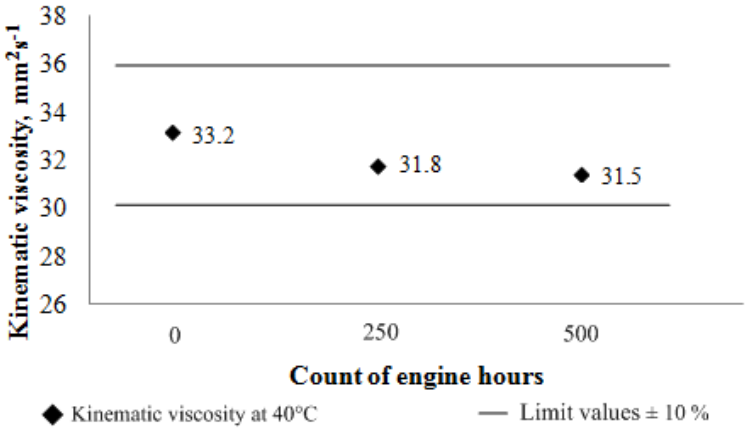

Figure 2. Kinematic viscosity at 40 °C during operating test.

Kinematic viscosity at 40 °C (Fig 2) is evaluated based on the positive or negative tolerance of the measured values in comparison with the value of new oil (at zero engine hours). Therefore, the kinematic viscosity at 40 °C of new oil must be evaluated. The deviation of kinematic viscosity at 40 °C is calculated by using the formula:

$$\Delta V = \frac{V_0 - V_{500}}{V_0} \cdot 100 \qquad (2)$$

where: ΔV is the deviation of kinematic viscosity at 40 °C (%); V_0 is the kinematic viscosity at 40 °C at zero engine hours ($mm^2\ s^{-1}$); and V_{500} is kinematic viscosity at 40 °C at 500 engine hours ($mm^2\ s^{-1}$).

The decrease of kinematic viscosity at 40 °C was calculated as follows: $\Delta V = 5.12\%$ according to Eq. (2), based on the value of new oil, $\nu_0 = 33.2\ mm^2\ s^{-1}$, and the value of used oil, $\nu_{500} = 31.2\ mm^2\ s^{-1}$. The decrease of kinematic viscosity at 40 °C does not exceed the limit of 10% which is prescribed for the UTTO (the limit of kinematic viscosity changes from the beginning of the operating test). Alias et al. (2009) evaluated kinematic viscosity at 40 °C of palm oil-based TMP ester (TMPE) and found the increase in kinematic viscosity after completing 400 hours, $\Delta V = 1.72\%$.

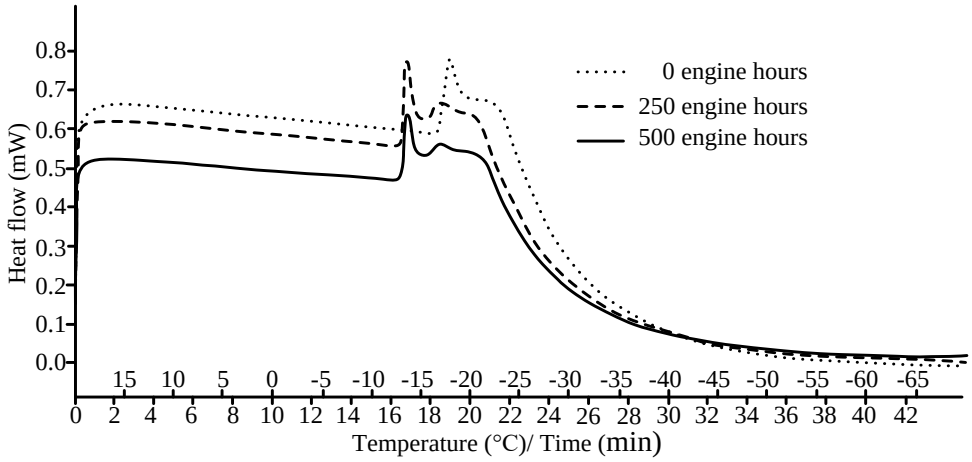

Figure 3. A comparison of oil samples by DSC.

DSC curves which correspond to a change of enthalpy due to thermal effects in the samples are shown in Fig 3. In the process of oil freezing and in the case of a new oil sample being used, we observed an exothermal peak at a temperature of -17.85 °C, corresponding to the pour point at which solidification occurs. Generally, the temperature for phase transition depends on chemical composition and on the crystalline structure of the materials. Since oil (at zero engine hours) is an amorphous matter, the point for the actual pour point takes place not only at one point, but in a range of temperatures which are shown in the graph (with an onset at -16.89 °C and an endset at -20.00 °C). In the case of oil at 250 engine hours, the peak temperature was -13.55 °C (with an onset at -13.08 °C and an endset of -14.75 °C). In the last sample, representing used oil at 500 engine hours, the temperature for the exothermal peak was almost the same as the previous results, -13.53 °C (with an onset at -13.04 °C and an endset at –14.78 °C).

CONCLUSIONS

Tribotechnical diagnostics use oils as media that help when it comes to obtaining information about processes and changes in the systems that they lubricate. If tribodiagnostics are applied properly and thoroughly, they result in significant savings in many areas; for example, they contribute to an increase in the lifetime of machines and equipment, to a decrease in the consumption of energy, and to a limiting of idle time (Kučera et al., 2013).

The DSC graph indicates that peaks corresponding to the pour point for worn out samples are almost identical, so we can infer that the difference between 250 engine hours and 500 engine hours is not especially significant. But we can see the difference between a new and worn out sample. The pour point (or freezing temperature) from both worn out engine oil samples increased by more than 4 °C in comparison with a new sample: -13.55 °C (at 250 engine hours) or -13.53 °C (at 500 engine hours) and –17.85 °C (for a new engine oil sample). We consider that oil wear has a negative impact on thermal stability and temperature when the phase transition (solidification) occurrance is higher. Our results for the pour point are considerably different from the figures that have been indicated by the oil's producer (Table 1); the pour point introduced into the producer's specifications is set at -48 °C. This difference may be caused by several factors (incorrect data being provided by the producer, inappropriate storage conditions, or other unknown factors such as, for example, thermal history). The figure should be verified by means of experimentation in the next phase of research.

After completing 500 engine hours the operating test for hydraulic oil was completed. In Table 3 the decreasing trends for oil additives can be seen. The biggest decrease in oil additives was observed with boron (B) and cadmium (Ca). Boron (B) content decreases from 83.34 mg kg^{-1} to 63.0 mg kg^{-1}, and cadmium (Ca) content decreases from 2,531 mg kg^{-1} to the 2,067 mg kg^{-1}. Boron is used as corrosion inhibiter and cadmium is used as a detergent additive.

Kinematic viscosity is one of the most important properties for the characterisation of lubricants and their transport properties, and is a measure of internal friction in a fluid (Alias et al., 2009). For this reason we need to remember the differences for kinematic viscosity during the operating test.

Experimental results deliver important areas of information from the point of view of oil degradation. The majority of tractors are subjected to conditions (especially during the winter) which can cause an undesirable phase transition of oil in hydraulic systems. It is necessary to further develop and improve oil flow by means of the correct operation of hydraulic equipment. The oil flow rate is important to the life of the hydraulic system.

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Vehicle in-use tyre characteristics evaluation during winter driving training

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Abstract. After the substantial number of fatalities in road traffic increase with the start of icy 2014–2015 winter conditions in Latvia drivers have been offered complimentary winter driving training. Having numerous drivers with their cars performing the same braking actions on restricted and safe test ground gave a good opportunity for evaluating driver skills and vehicle in-use tyre condition. Tyre age and tread depth were measured and driving instructions have been given to more than a thousand participants. Instrumented braking and manoeuvring measurements were made for thirty cars. The equipment included GPS receiver and decelerometer. Measurement results have been compared against physical observations and hints given by professional driving instructors. The comparison indicated that although the suggestions given to drivers by professional instructors clearly contribute to safer driving, the visual evaluation without measuring may lead to various errors in messages conveyed to drivers. The most questionable statement was about the influence of the vehicle mass on braking distance. The investigation did not show essential vehicle mass influence on tyre grip and correspondingly on braking characteristics. Tyre tread depth effect on braking performance demonstrated the relevance of technical requirements for winter tyres. The advantage of using public training for winter tyre in-use experiments is lower cost for getting tyre samples with various technical conditions. The disadvantages are the different skills of car owners, the need for individual instructions and the necessity to reckon with the training format, the limited choice of the testing variables and substandard conditioning of the road surface. The tests gave valuable information for further training sessions and coming winter tyre tests.

Key words: traffic safety, braking, tyre grip.

INTRODUCTION

The start of 2014–2015 winter season on Latvian roads turned out tragic. Due to fast changing weather conditions, the road maintenance was not capable of fast and effective road surface treatment. Many drivers failed to act adequately on icy highways and rural roads. During two months with winter conditions the number of deaths compared to the previous year almost tripled. Latvian Road Traffic Safety Directorate decided to offer complimentary winter driving training hoping both to increase skill levels of the participants and to draw more attention to safer driving on icy roads. Area at state owned car race track Bikernieki in Riga was offered for training.

Equipping test vehicles with different tyres having various technical condition is labour and resources intensive. Renting and conditioning a safe test ground is quite costly and there are no many test grounds on offer in the region. Therefore having numerous drivers with their cars in constrained area performing the same braking and manoeuvring actions seemed a worthy chance to perform research on certain driving skills and in-use tyres grip on icy and snowy surface.

While observing the training process certain misinterpretation of vehicle dynamics by professional driving instructors has been noted. Although absolute majority of practical advice given to the drivers was accurate, the theoretical interpretation of driving mechanics rose interest in how proficient trainers came to certain theories. The most questionable were statements about vehicle mass influence on braking distance. This supplemented the initially planned subject of the research of finding updated in-use tyres braking characteristics variations on winter road surfaces by analysis of winter driving training blunders.

The history of winter tyres and road grip improvement in snowy and icy conditions goes back to 1930-s. From 1970-s many countries have introduced laws relating to mandatory use of winter tyres during certain winter period or meeting specified winter conditions. However various countries having different weather conditions and temperature range variations, having or lacking mountainous regions or many roads with steep gradient, face different winter problems and therefore the legislation varies from country to country. Currently the usage of winter tyres with tread depth at least 4 mm is mandatory in Latvia from 1st December to the end of February; studded tyres are prohibited from 1st May till the end of September.

The road and tyre friction has a great impact on traffic safety (Wallman & Åström, 2001). To provide ground for legislation change and to develop and promote new tyre models, a lot of research has been done in many countries. The tyre friction on ice and snow is tested in tribology laboratory experiments (Skouvaklis et al., 2012); (Ella et al., 2013), on special tyre traction testing machines, (Gießler, 2007), on full scale conditioned test grounds (Hjort & Eriksson, 2015) and on winter roads (Walus, 2016). Since there is no tyre production in Latvia, the most interest is in the process of deterioration of tyres and performance of used winter tyres on snowy and icy roads.

A leading Swedish National Road and Transport Research Institute VTI is located in Östergötland region where weather conditions are quite similar to Latvia. Therefore apart from doing some local tests it has been always worth to follow the VTI findings in traffic safety. In the most recent research (Hjort & Eriksson, 2015) on tyre test equipment and on properly prepared test grounds studies how the road grip on ice and snow for different types of winter tyres degrade by age and degree of wear, how Nordic and European, budget and premium brands produce different results. Investigation of the correlation between grip on rough ice of winter tyres and their age, tread depth and tread rubber hardness (Nordström, 2003) emphasizes the advantage of studded and new tyres but due to very few tyres with tread below the minimum does not show the consequences of ignoring the law. Although new tyres show better performance (Nordström, 2004), contrary to the opinions of tyre advisors, investigation of the influence of tyre age in the range from 5 to 15 years did not prove tyre grip change.

The research objectives were to supplement and update tyre in-use grip characteristics in winter conditions, to develop tyre characteristics evaluation techniques without costly test ground conditioning, to test the hypothesis about car braking intensity

dependence on vehicle mass and to comprehend how this hypothesis has emerged. The scope of the research was limited by the cars participating in the training and does not represent the full range of in-use tyres, the findings were limited by the weather and road surface conditions available, no tests were performed on smooth ice or in deep snow.

MATERIALS AND METHODS

The driver training was done according to organizers plan. To benefit from having more trainees, the number of activities by each vehicle was limited to one straight braking and four to six braking with manoeuvring trials. The participants were asked to accelerate to 50 km h^{-1} and from the line marked by lying traffic cones in a corridor to perform a full emergency braking to standstill (Fig. 1).

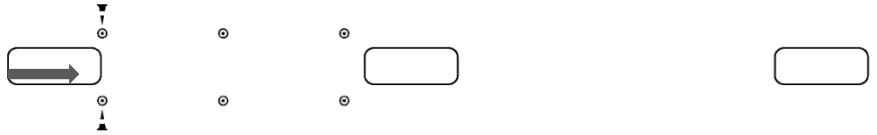

Figure 1. Braking schematics showing car positions from the start of braking to standstill.

To perform vehicle manoeuvring and braking trials the braking section was supplemented with cones displaced closer than the vehicle stopping distance (Fig. 2), requiring a manoeuvring similar to braking with line change. The participants were asked to accelerate to 50 km h^{-1} and from the line marked by lying traffic cones to stop the vehicle without hitting the traffic cones.

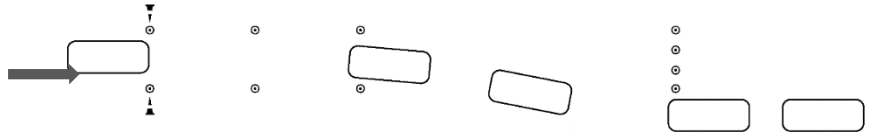

Figure 2. Manoeuvring with braking schematics, showing car positions from the start of braking to standstill.

The trainers evaluated braking distance for braking trials and the ability of drivers to avoid cones and return into driving direction after manoeuvring effort. To calculate the tyres in-use grip characteristics and to appraise the trainers' capability of decision making based on visual evaluations for each participant group, one car was equipped with VBOX GPS meter Driftbox logging GPS and speed data every 0.1 s and Inventura decelerometer XL Meter logging longitudinal and lateral acceleration every 0.05 s. Logged data were downloaded to Excel and processed and visualized using Excel VBA code and Uniplot software. The braking start positions, vehicle trajectories and braking distances were calculated from GPS data using flat Earth model, braking speed found from GPS speed recordings and accelerations calculated both from speed recordings and decelerometer logs. Since all trainees did not fully comprehend the tasks, and several of trainer's conclusions dissipated to trainees needed to be checked, some planned tests

were done on the same training sectors while the trainees groups changed and received instructions.

In total the measurements were done for ten training days until the training was interrupted by too warm weather conditions. Measurements were done for 30 cars, including two cars for planned tests, 200 runs measured, 69 straight braking events and 131 braking events with manoeuvring trials. Various car models equipped with appropriate size winter tyres have participated in the tests: Audi 80, A4, Q3, Q7, BMW 3 and 5 Series, Chrysler PT Cruiser and Voyager, Citroen C2, Dodge Caliber, Honda Accord and Civic, Lexus IS200, Mazda 6 and 626, Mercedes-Benz C Class, Opel Astra and Zafira, Seat Ibiza, Subaru SVX, Toyota Land Cruiser, Volvo S60 and XC70, VW Golf 7, Tiguan and Tuareg. Only cars equipped with ABS system were selected for the tests. For each vehicle the tyres year of production was determined and minimal tread depth recorded. The training ground was covered with 3 to 5 cm of packed snow with patches of ice underneath, the temperature was between -7°C and +3 °C.

The measurement data processing algorithm was formed in accordance to the results obtained from the passive experiment strategy. The braking characteristics and trajectories in relation to the acceleration line of each individual car for every measured run are shown on Fig. 3.

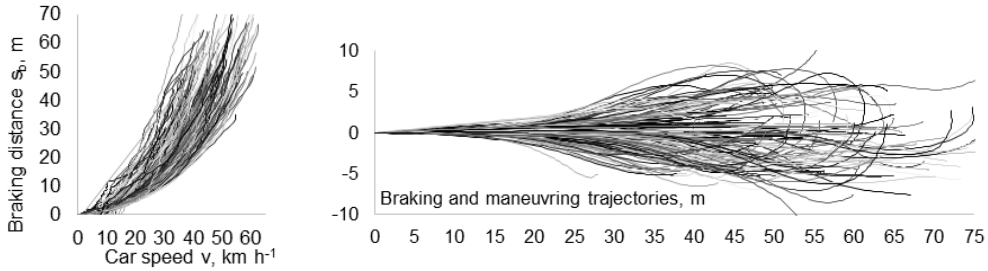

Figure 3. Braking distance (left) and trajectories (right) for all cars measured.

Both graphs show a huge variety in results. The braking distance from 30 km h^{-1} varies almost four times, not all cars have braked to standstill, many cars have not reached the target braking start speed 50 km h^{-1}, the trajectories of manoeuvres show essential discrepancy from the task, the loss of car control and running off the imagined road lane. Braking distance variations from 40 km h^{-1} can be seen on the right side of Fig. 4 while on the left side – the number of cars performing braking on the given stretch of the training ground.

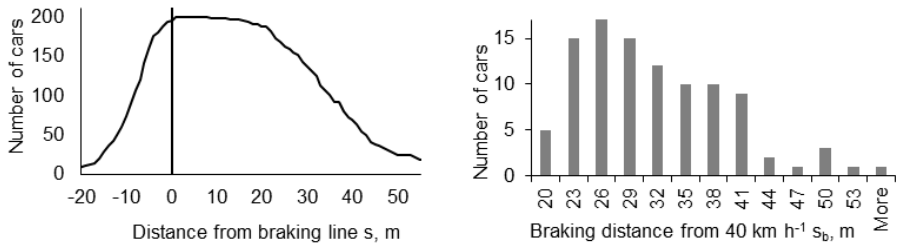

Figure 4. Braking start poin (left) and braking distance distribution from 40 km h^{-1} (right).

Both graphs show that more than 70 metres of the test ground have been used for braking and drivers have started braking even more than 20 m before the braking line. Although the braking test grounds were changed to other location and conditioned for the training almost every day, the number of cars performing training influenced the state of the road surface. The snowy surface of the training area was tamped by snow tractor or bulldozer and overrun by test driving. After the training sessions the condition of the training ground may be characterized by Fig. 5 where average deceleration of the cars has been plotted along the braking track. The average deceleration change by nearly 1 m s^{-2} or by over 50% results from the more intensive usage of the area between braking cones than the further area where both straight braking and avoiding the obstacles from both sides evens the road load. On the left graph the deceleration values are given both for braking without and with manoeuvring trials, indicating that on the most slippery braking ground stretch there was no essential difference between the test modes while manoeuvring along the obstacle cones was done at the expense of some braking efficiency.

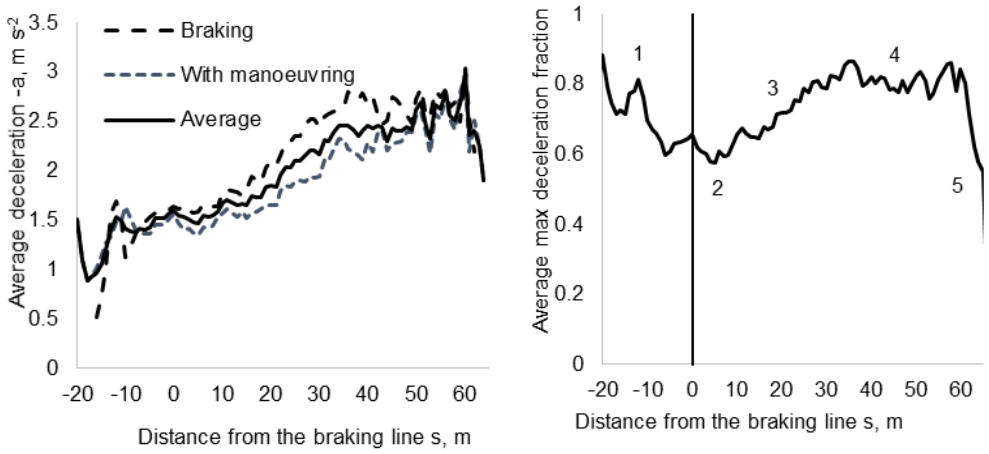

Figure 5. Average deceleration (left) and the fraction from the maximum deceleration (right) along the braking track.

If the braking efficiency along the track is characterized by the fraction from the maximal deceleration of each individual run then quite clearly five zones can be seen on the right graph of Fig. 5: 1 – early braking on less slippery surface; 2 – braking on the most slippery stretch between the traffic cones; 3 – start of the manoeuvring; 4 – less hurt road surface with higher grip; 5 – end of braking. Due to the changing road friction along the test track, the braking intensity calculations were made for separate track parts characterized by the distance from the intended braking line marked by traffic cones in increments of 1 m and 5 m.

Distribution of braking intensity (number of cars braking at given intensity range) is shown on Fig. 6. It can be seen that almost at every braking track section wide range of braking intensity is present. Nevertheless the most represented area is the same as for the zone of average values. The graphs indicate that along the whole braking track emergency braking may have not been applied and the maximum deceleration values are

more informative than average values, therefore braking results have been evaluated by maximum deceleration in segments of distance and in segments of every 10 km h^{-1}.

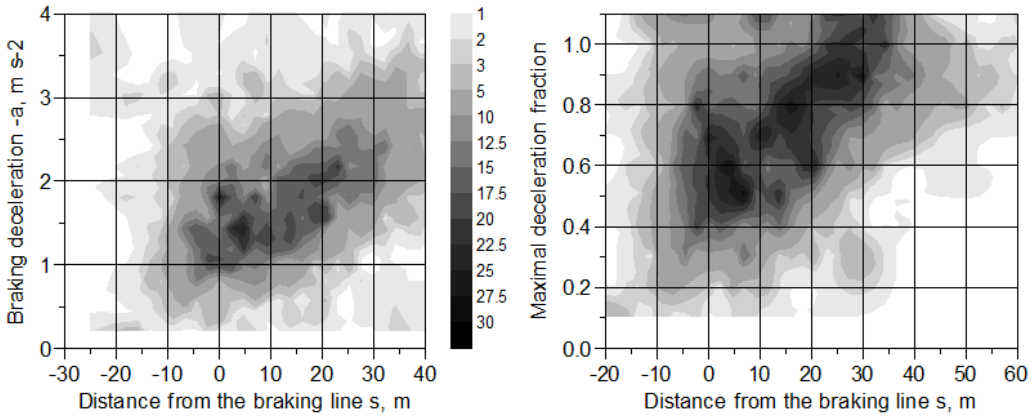

Figure 6. Distribution of the braking deceleration (left) and the fraction from the maximum deceleration (right) along the braking track.

Driver training started by theoretical instruction. Among other practical and correct driving tips the trainers developed the hypothesis that the car braking distance for heavier cars on snow is essentially longer than for lighter cars. Appreciating the practical experience of the instructors it was decided to check the stated hypothesis.

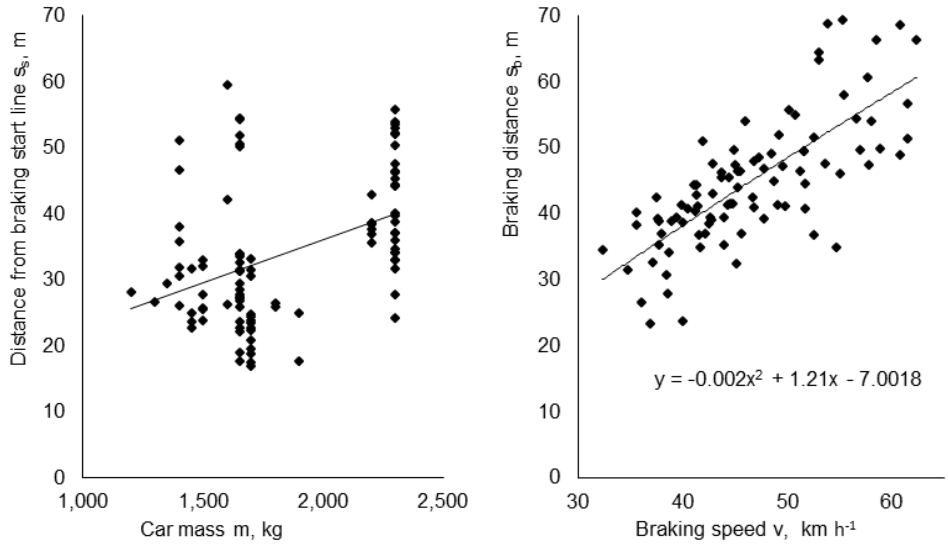

Figure 7. Confusing graphs with trendlines from the training: braking distance dependancy on mass (left) and braking distance dependancy on braking commencement speed (right).

The draft results of the mass influence is shown on the left of Fig. 7 where car stopping position that can be fixed by trainers allegedly indicates that heavier cars may have some ten extra meters of braking distance from 50 km h^{-1}. Other draft graph shown on the right of Fig. 7 suggested that braking distance on snow is almost linear to the braking speed. Both confusing findings have been analysed along with tyres in use influence on braking efficiency in snowy conditions.

RESULTS AND DISCUSSION

The car in-use tyre characteristics have been evaluated by measuring car braking speed, deceleration and GPS coordinates on uneven snowy road segments during winter driving training at the dedicated test track area. The influence of tyre tread depth, tyre age, using studs and vehicle mass on braking distance, maximal deceleration and average deceleration in speed and distance intervals have been analysed.

Tyres with equal tread depth for the tested cars have been manufactured in five year range and no essential difference in braking characteristics on snowy surface has been found for this short period and the limited number of cars. Only six cars equipped with studded tyres have been used for training. The studs did not demonstrate advantage on snowy surface but due to the small number of tests the results have not been further analysed and the cars have been excluded from the analysis of tyre tread depth influence on braking characteristics.

Braking distance graphs from 30 and 45 km h^{-1} are shown on Fig. 8. Cars that did not fully stop or braking intensity reduction in speeds below the test speed has been noticed in the braking or manoeuvring process have been excluded from the results, but even the limited size of the test sample shows essential difference in braking intensity with tyre tread depth below 6 mm.

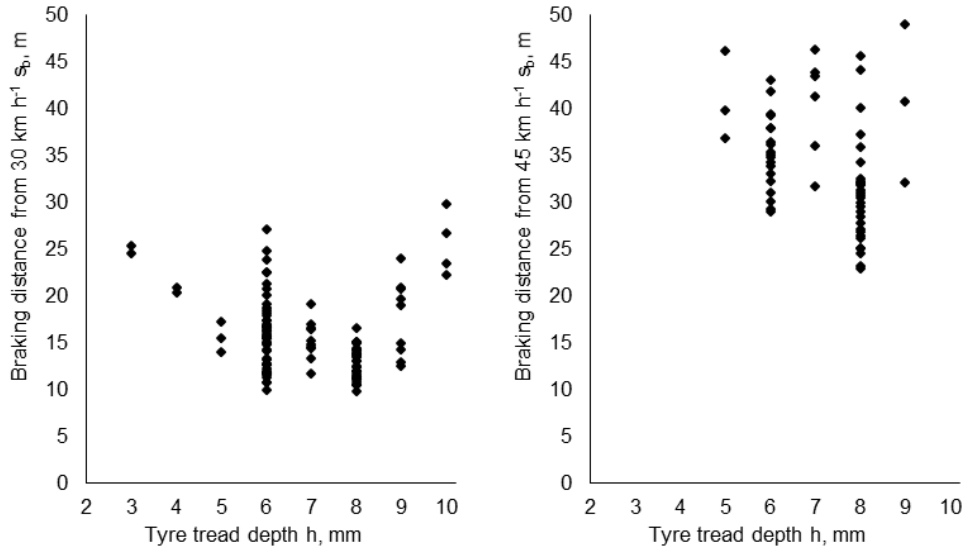

Figure 8. Braking distance from 30 km h^{-1} (left) and 45 km h^{-1} (right).

Plotting results for braking maximal deceleration within limited speed or distance ranges on Fig. 9 allows the usage of data from cars that did not fully stop, diminished the braking force during braking or manoeuvring. The results confirm the same trend of strong influence of tyre tread depth on braking intensity.

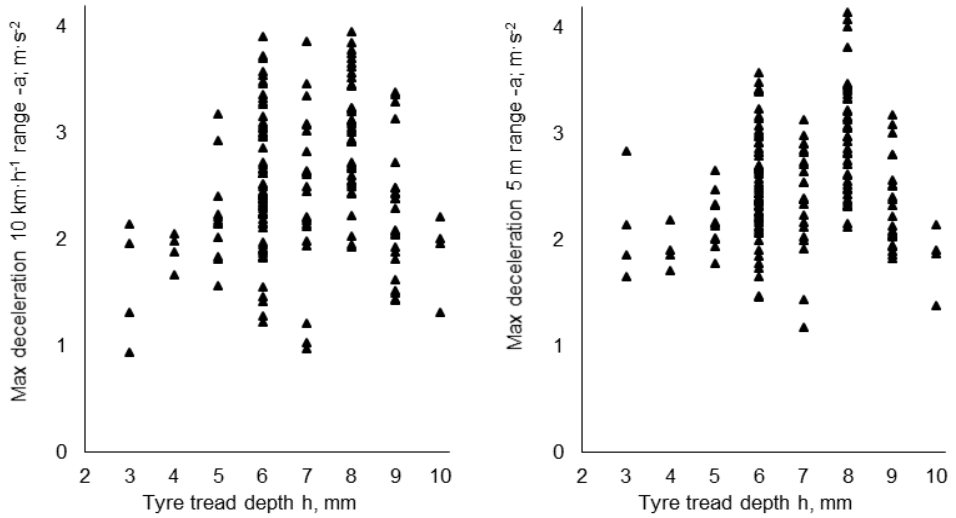

Figure 9. Braking maximal deceleration within 10 km h^{-1} range (left) and 5 m range (right).

Even more regular results have been obtained from the analysis of maximal deceleration achieved on particular distance from the braking line shown on Fig. 10. The results approve the tread depth requirements for winter tyres in use and suggest that mounting tyres close to minimum requirements at the start of winter season compromises with safety on snowy roads.

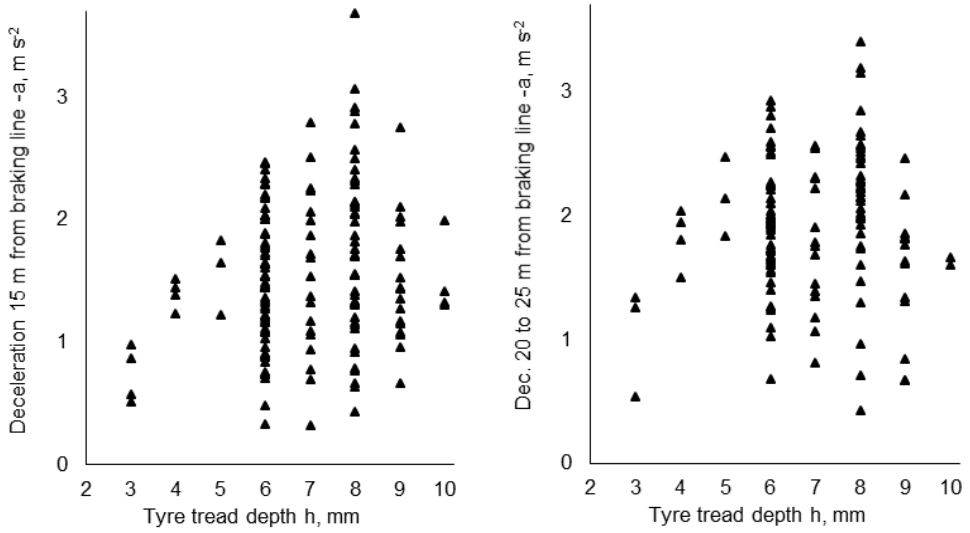

Figure 10. Braking deceleration 15 m (left) and 20 to 25 m (right) from braking line.

The recorded influence of tyre tread depth on maximal deceleration in the region from 3 mm to 8 mm exceeds the trend shown in publications (Nordström, 2004, Jansen, 2014). This may be caused by the small number of runs with tyres with low and high tread depth.

Several tests and calculations have been done to test the hypothesis of car mass influence on braking intensity. The main graphs for these tests are shown on Fig. 11.

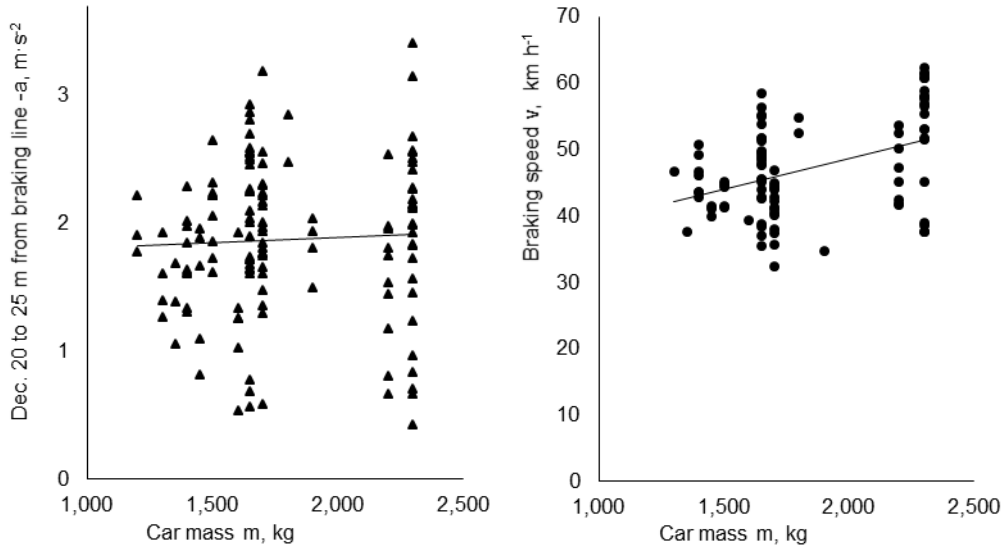

Figure 11. Car mass influence on braking intensity: deceleration (left), braking speed (right).

Although car mass may influence coefficient of friction (Skouvaklis et al., 2012; Gießler, 2012), no braking intensity decline has been recorded during the tests, including several separate tests where the same car has been tested with driver only and fully loaded with passengers. The perceptions of driving trainers about problems with heavier cars were clarified when car acceleration and braking speed was plotted against the car mass (Fig. 11, right). The proportion of all-wheel drive cars among the lighter cars is smaller than among the heavier cars. Due to safety reasons limited car acceleration distance has been offered for the trainees therefore cars with better tyre grip accelerated to higher speeds. This also explains the confusing graph on Fig. 7 right, where braking from higher speed is compensated by higher tyre grip, therefore the graph may have this form only when limited acceleration distance is available.

CONCLUSIONS

The vehicle in-use tyre characteristics evaluation in winter conditions can be done using events like driver training without setting up the training ground and specifically planning the experiments but the results obtained may be limited to existing road surface and weather conditions, tyre condition and models used by the participants and activities scheduled. Having less conditioned and less uniform braking ground for tests can be partially compensated by performing separate analysis on road sections with dissimilar

grip, evaluating the maximal braking deceleration achieved in speed and distance intervals.

For the snowy winter conditions tyre tread depth has essential influence on road grip. Thread size below the legally allowed limit of 4 mm may reduce braking efficiency more than three times if compared with tyres with full tread.

Performing braking trials without instrumentation even by professional trainers may lead to incorrect judgements. The hypothesis of the driving trainers that heavier cars have essentially longer braking distance in slippery conditions was rejected. No essential mass influence on braking intensity was found and the inaccurate hypothesis has been originated from the wrong evaluation of initial braking speed.

Experience with tyre characteristics evaluation during winter driving training will help setting up more fundamental tyre grip tests in winter conditions.

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Detection of changes in the water, blackcurrant- and raspberry juice infrared spectrum in the range 2,500−4,000 cm^{-1}

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Abstract. This research was conducted to develop a method for the establishment of changes in absorbance that occurs in the OH stretching vibration region 2,500−4,000 cm^{-1} of FT-IR transmission spectra of water and juices. With the methodology described in this paper, a signal-to-noise ratio was obtained, which allows to measure the reproducibly of the FT-IR transmission spectra of water and juices. The article points out some important aspects of how the spectra of ultrapure water, blackcurrant- (Ribes nigrum L.) and raspberry (Rubus idaeus) juices were measured in the OH stretching vibration region 2,500−4,000 cm^{-1}. The graphical comparison of raw spectra illustrates that the differences which occur in the spectra of water and juices are hard to differentiate. For the clear distinction of differences in spectra, there are four hidden peaks identified in the analysis of spectra by using the deconvolution method. There are clear differences identified in the comparison of percentages of the hidden peaks of spectra in the areas of ultrapure water, blackcurrant and raspberry juice. Repeated measurements and analysis of spectra provide reproducible results. It was established that the developed method can be used for the detection of changes in the FT-IR transmission spectra of water, juices and aqueous solutions with low molar concentrations of additives. The distinction of changes in the spectra is a precondition for research of the clustered structures of water.

Key words: water structures; clustered water; FTIR; hidden peaks; raspberry juice; blackcurrant juice.

INTRODUCTION

The study of water structure in biological systems has been a research topic for more than half a century (Plumridge & Waigh, 2002; Laurson & Mäeorg, 2015). Scientists have used different spectroscopic methods to reveal water structure: NIR (Bharmoria et al., 2014), Raman (Walrafen, 1967), IR (Falk & Ford, 1966) NMR (Dillon & Dougherty, 2003; Conte, 2015). When water structure changes under the different factors affecting it, the number of hydrogen bonding may increase or decrease. The changes in water structure also change the infrared radiation interaction with water and will, therefore, cause changes in the infrared spectra. The strength of hydrogen bonds decreases with the increase of bonds in length which depends on the geometry of water clusters and is easily affected by the environment (Jeffrey & Saenger, 1991; Calhorda, 2000). In order to investigate water structure, any technique to prevent its alteration is

needed. Infrared spectroscopy is well suited for this kind of research in biological systems. Walrafen studied the structure of pure water by infrared spectroscopy and in his model described that the broad OH vibration maxima in the range of 3,000−3,800 cm^{-1} could give four deconvoluted peaks located around ~ 3,245, ~ 3,420, ~ 3,520, ~ 3,620 cm^{-1} and weak fifth centred near 3,060 cm^{-1}(Walrafen, 1972). These peaks were assigned as non-hydrogen-bonded OH stretching peaks 3625−3645 cm^{-1}; 3,540−3,555 cm^{-1}, hydrogen-bonded OH stretching peaks 3,395−3,420 cm^{-1}; 3,215−3,220 cm^{-1} and fifth component 3,050−3,090 cm^{-1} arising from Fermi resonance between the overtone of the bending vibration at ~1,650 cm^{-1} and coupling main component of 3,215−3,220 cm^{-1} correspondingly (Walrafen, 1972). The researchers have thereafter used changes in the absorption spectra in the aforementioned regions in order to describe changes in the clustered structure of water. The high molar concentration of additives (inorganic salts, urea, sugars etc.) in aqueous solutions causes clearly distinctive changes in spectra (Afrin et al., 2014; Bharmoria et al., 2014; Mafy et al., 2015). In biological systems the concentration of different dissolved substances is a few percent (Despa, 2005), therefore, differences in the infrared spectra of the fundamental vibration region of pure water are low as compared to the spectra of the biological system. The aim of the paper is to identify the reproducibility of small changes by eliminating all the factors affecting the measurements.

MATERIALS AND METHODS

High-quality ultrapure water was obtained by the Milli-Q® Advantage A 10 Water Purification system (Merck Millipore). The resistivity of ultrapure water at 25 °C was 18.2 MΩ·cm and TOC (total organic carbon) 3 μg l^{-1}. The water sample was held in a closed glass flask. The blackcurrant *Ribes nigrum L.* (Pamjati Vavilova) and raspberry *Rubus idaeus* (Novokitaivska) juices, which were employed in the studies, were pressed from defrosted berries of the 2015 year harvest by a belt press Voran EBP 500, pasteurized at Polli Horticultural Research Centre PlantValor of the Institute of Agricultural and Environmental Sciences, the Estonian University of Life Sciences, and the samples were preserved in closed polypropylene tubes at 7 °C. The yield of blackcurrant juice (without previous enzyme treatment) was 53.8% and the yield of raspberry juice 64.0%. The content rated values of total sugar and content of soluble solids were based on the published research data of the four year average of raspberry (Novokitaivska) 10 ± 2°Brix (Arus et al., 2008) and five year average of blackcurrant (Pamjati Vavilova) 15 ± 1°Brix (Kaldmaee et al., 2013). The content of soluble solids in the homogenised juice samples was measured by a Pocket Refractometer 'ATAGO' PAL- α at 22 °C.

Infrared spectra were measured at a resolution of 4 cm^{-1} with the Perkin Elmer Spectrum BXII FT-IR system equipped with a DTGS detector in the absorbance mode with 32 scans for each sample in the range 2,500−4,000 cm^{-1}. The homemade reduced sample compartment was flushed continuously with N_2. Spectra acquisition started after 3 minutes from the cleaning process of the sample compartment with N_2, i.e. after having invariably checked the background noise. The spectra were acquired by the application of CaF_2 windows (thickness 4 mm; diameter 32 mm). A 1.2 μl proportion of the sample was applied on the CaF_2 window by using a 10 μl Hamilton syringe. The sample was spread on the window with an even layer and covered with another window. The edge

of the windows was tightly framed with two layers of Parafilm® tape to stabilise the layers of the fluid. The thickness of the layer between the windows was 1.5 μm which was calculated by applying the volume formula for a cylinder. All the measurements were performed at a room temperature in the range of 22−24 °C. Good reproducibility reveals that 2 °C temperature variations of the measurement interval do not affect the result of the measured FT-IR spectra. At least three measurements of each sample were carried out for the reproducibility of the measurement. For the deconvolution of spectra OriginPro 2016 Sr1 b9.3.1 273 (OriginLab Corporation) a software peak analyser tool (Goal: Fit Peaks Pro; Anchor Points Finding Method: 2nd Derivative; Fitting Function: Line; Current number of Peaks 4; baseline parameters and peak centres are released) was used. All the spectra before the deconvolution were normalised to 0−1.

For precise measurements and the detection of small changes in infrared spectra, all the sources of noise and interferences should be detected and eliminated. One of the serious sources of noise is the background spectrum which contains signals from water, carbon dioxide and alkane. The above background spectrum is generally subtracted from the spectrum of the sample. Since the atmosphere in the laboratory is likely to change, the subtraction of the background spectrum does not prove extremely precise and some noise will still be registered.

The noise disturbs the detection of small changes in spectra and the correct deconvolution. In order to decrease noise, the sample compartment should be flushed with dry nitrogen. Nevertheless, flushing as it is recommended by spectrometer companies fails to appear sufficiently efficient. Therefore, a special N_2 purgeable tube was manufactured and tightly installed in the sample compartment of the spectrometer. It may be followed in the comparison of the background spectrum measured in the common laboratory environment versus the background spectrum measured in the environment purged with N_2 (using installed tube), that in the water vapour absorption region 3,400−4,000 cm^{-1} the peaks decrease up to 80−90%, as shown in Fig. 1. This level of noise is acceptable for the measurements of small spectral changes in the OH stretching vibration region and thus is there no need for an expensive spectrometer with closed and inert gas filled optical system.

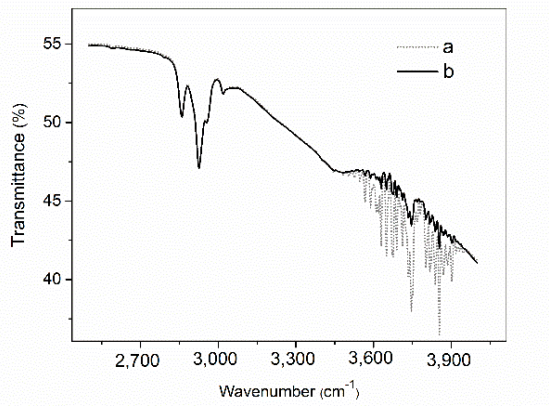


Figure 1. Comparison of the background spectra: a) ordinary air in room, b) N_2 flushed special sample compartment.

RESULTS AND DISCUSSION

Fig. 2 shows that the infrared spectra of ultrapure water, blackcurrant- and raspberry juice have a joint peak absorption maximum at 3,404 cm^{-1}. Moreover, all the spectra reveal an isosbestic point (the i.e. common point where spectral bands cross) at 3,590 cm^{-1} which suggests the existence of differences in the spectra (Afrin et al., 2014; Walrafen et al., 1986). To the opposite, when the crossing points between the spectra are missing, this would mean that the absorption spectral band profiles are the same, and no changes occur in the spectra.

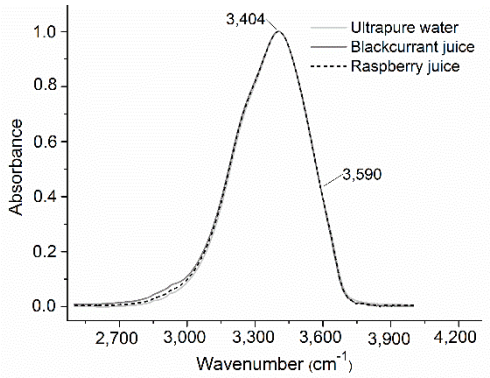


Figure 2. FT-IR spectra of ultrapure water, blackcurrant juice and raspberry juice in the range 2,500−4,000 cm^{-1}.

Fig. 3 shows that the observed lines of spectra do not overlap at the beginning and the end of spectra, but the order of the lines changes at 3,590 cm^{-1}, thus indicating the presence of different interactions between juice components and water molecules. These changes would be difficult to detect should the spectra stand separately. It is common knowledge that the strong absorption bands that belong to the water stretching mode at 3,404 cm^{-1} consist of four individual maxima (Venyaminov & Prendergast, 1997) which are visualised after the deconvolution of spectra.

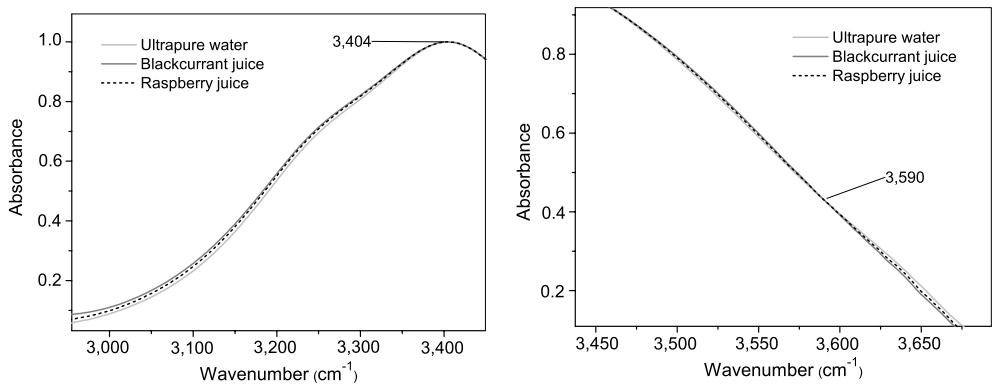


Figure 3. The joint peak absorption maximum at 3,404 cm^{-1} and isosbestic point in the 3,590 cm^{-1} of FT-IR spectra of ultrapure water, blackcurrant juice and raspberry juice in the range 3,000−3,700 cm^{-1}.

The deconvoluted FT-IR spectra of ultrapure water and juices are shown in Fig. 4, the maximum of bands corresponds to area A1: 3,100−3,220 cm^{-1}, A2: 3,240−3,265 cm^{-1}, A3: 3,430−3,440 cm^{-1} and A4: 3,580−3,590 cm^{-1}. As deconvolution was performed without restriction, it mean baseline parameters and peak centres was released, as expected sifhts in the maximum of bands locations and result is accordance with Walrafen research. (Walrafen, 1972). Fig. 5 shows that the researched substances are compared to each other whereas the integrated hidden peaks (A1, A2, A3, A4) area of the deconvoluted FT-IR spectra are all different. The hidden peaks areas A2, A4 of juices are higher and A1, A3 lower than ultrapure water in the same peak areas. The error bars in Fig. 5 show that deviations in the measurements of spectra are very low and fail to pose any impact on the results.

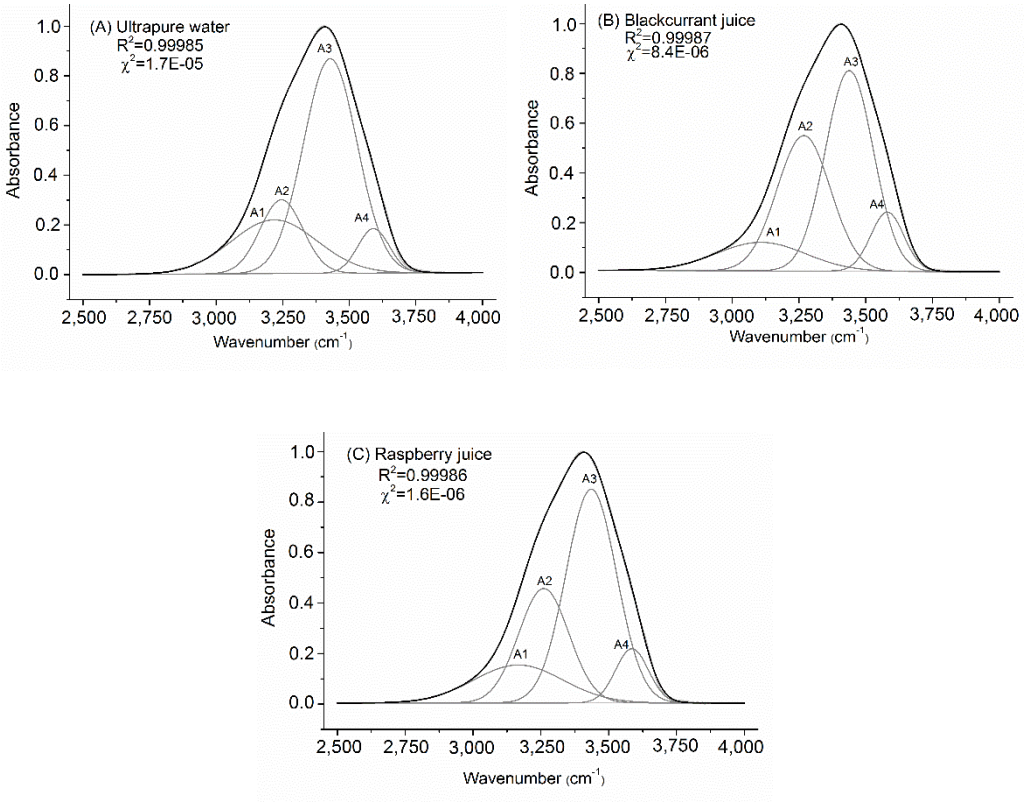
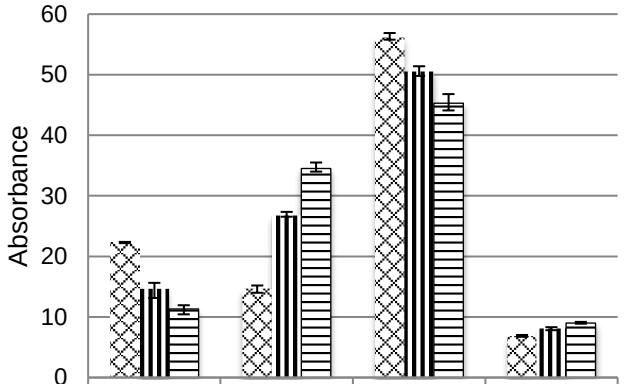


Figure 4. Comparison of deconvoluted FT-IR spectra: a) ultrapure water, b) blackcurrant juice and c) raspberry juice in the range 2,500−4,000 cm^{-1}.



	A1	A2	A3	A4
Ultrapure water	22	15	56	7
Raspberry juice	15	27	50	8
Blackgurrant juice	11	35	45	9

Figure 5. Comparison of integrated hidden peaks (A1, A2, A3, A4) area in percent of the total area of the deconvoluted FT-IR spectra ultrapure water, blackcurrant juice and raspberry juice in the range 2,500−4,000 cm^{-1}.

CONCLUSIONS

It has been demonstrated that by using the modified sample compartment, which has been purged with N_2, it is possible to measure reproducibly the infrared spectra of water and juices in the OH stretching region 2,500−4,000 cm^{-1}. The deviations that occur in the spectra of water and juices are hard to discern. By using the deconvolution method in analysing the spectra, a clear distinction may be observed in the deviations within the spectra. There are clear deviations in the hidden peaks areas of ultrapure water, blackcurrant and raspberry juice whereas it refers to the deviation in the structure of water in juices. The method developed can be used for the detection of changes in the FT-IR transmission spectra of water, juices and other aqueous solutions with low molar concentrations of additives.

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Support scheme for CHP and its sensitivity on heat wasting

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Abstract. This paper describes basic principles of the CHP, advantages and disadvantages, technologies. In first part of article are described principles of CHP from the viewpoint of the energy customer. Second part describes basic Directives by the European Commission on promotion CHP and third part the most important part focus on the sensitivity of primary energy saving (PES) on outputs especially heat wasting.

Key words: Combined heat and power, cogeneration, primary energy savings, primary energy, heat wasting, efficiency, support.

INTRODUCTION

Combined heat and power production (CHP) also known as cogeneration is the simultaneous production of electricity and heat from a single fuel source, such as coal, natural gas, biomass, waste heat, biogas, oil and etc. High efficient CHP is one of supported technology which can help to achieve the 20-20-20 goals the third target climate change and energy suitability. This ambitious European target means a 20% increase in energy efficiency and a 20% reduction of CO_2 emissions and a 20% renewables by 2020.

The main advantage is that the CHP systems are able to recover waste heat produced from the generation of electricity. That's why cogeneration has higher efficiency (Flin, 2010) than separate production of electricity and heat. The best example how to explain the principle of CHP is to look at it from a viewpoint of an energy customer. See Fig. 1 where principle separate production of electricity and heatis described. As we can see an overall efficiency of 58% is for separate production.

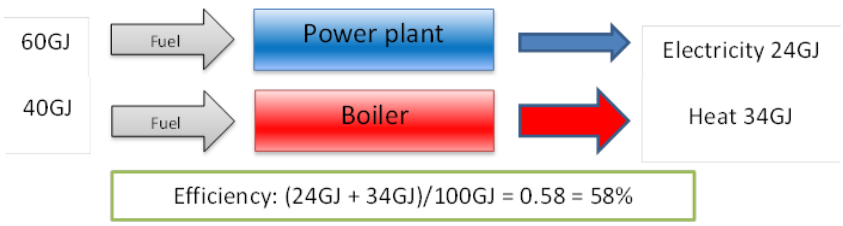

Figure 1. Separate production of electricity and heat.

The same example is but with CHP system; see Fig. 2 with an overall efficiency of 85%.

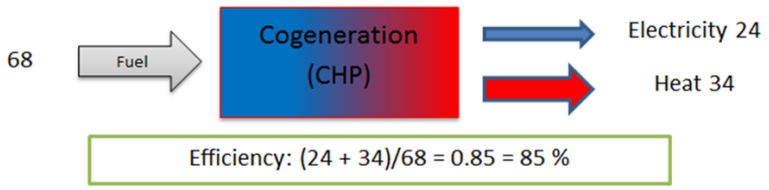

Figure 2. Cogeneration = simultaneous production of electricity and heat (Nedela & Nedela, 2013).

As you can see in the both figures the first advantage is that for same necessary units of energy (electrical energy and heat) for the end customer we will need less fuel units = less primary energy. Inputs and outputs are re-calculated for the same energy units. If we have less fuel units and less distribution of production losses then we will have also lower emissions to the environment in particular CO_2. So these are some of the advantages of CHP.

LEGISLATION

One of the most important Directives of the European Parliament and of the Council was 2004/8/EC on the promotion of cogeneration based on a useful heat demand in the internal energy market and amending Directive 92/42/EEC. This directive shows potential for use of cogeneration as a measure to save energy. We can also find here a new important definition of high-efficiency cogeneration that provides primary energy savings calculated according to equation (1) of at least 10% compared with the references for separate production of heat and electricity, production from small scale and micro cogeneration units (defined by 1 MWe installed capacity) that must provide primary energy savings (that means higher than 0%) may be to qualify as high-efficiency cogeneration (EC Directive, 2004). The issue of PES is briefly described in paper (Nedela & Nedela, 2013).

PES calculation (EC Directive, 2004):

$$PES = \left(1 - \frac{1}{\frac{CHP\ H\eta}{REF\ H\eta} + \frac{CHP\ E\eta}{REFE\eta}}\right) \cdot 100\% \qquad (1)$$

where: *CHPHη* is the heat efficiency of the cogeneration production defined as annual useful heat output divided by the fuel input used to produce the sum of useful heat output and electricity from cogeneration. *RefHη* is the efficiency reference value for separate heat production. *CHP Eη* is the electrical efficiency of the cogeneration production defined as an annual electricity from cogeneration divided by the fuel input used to produce the sum of useful heat output and electricity from cogeneration. Where a cogeneration unit generates mechanical energy, annual electricity from cogeneration may be increased by an additional element representing the amount of electricity which

is equivalent to that of a mechanical energy. *Ref Eη* is the efficiency reference value for separate electricity production.

In 2012 the new Directive 2012/27/EU of the European Parliament and of the Council on energy efficiency, amending Directives 2009/125/EC and 2010/30/EU and repealling Directives 2004/8/EC and 2006/32/EC, that is mostly about energy efficiency repeal the Directive 2004/8/EC and also sets important duties to Member States (Article 14) carrying out and notifying to the Commission a comprehensive assessment of the potential for the application of high-efficiency cogeneration and efficient district heating and cooling. This information will be important for the investors and will help to develop a whole CHP sector.

Next important material is Guidelines on State aid for environmental protection and energy 2014–2020 (EC Guidelines, 2014). This material follows a policy Framework for climate and energy in the period from 2020 to 2030, where are new pillars of the 2030: greenhouse gas emission reduction, increase amount of renewable energy and energy efficiency. For cogeneration and district heating and cooling is there special part that described support scheme for new application of CHP.

SENSITIVITY OF PES PARAMETER

The PES parameter compares CHP with reference separate heat and separate electricity production and it is the most important parameter which qualifies if the CHP producer can receive some state support. This state support must be in line with Guidelines on State Aid for Environmental Protection and Energy for actual period (now 2014–2020).

Fig. 3 shows a basic CHP scheme. Input is a fuel energy which goes into a gas turbine and outputs are electrical energy and useful heat.

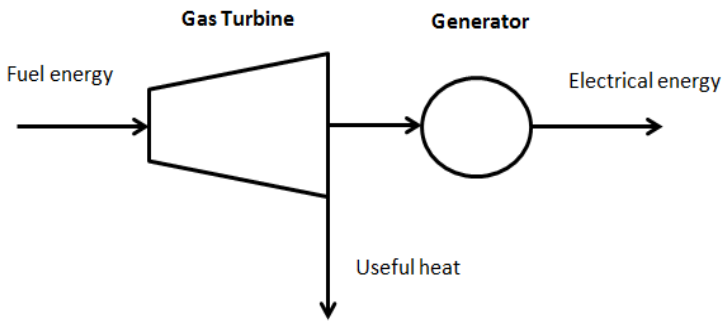

Figure 3. CHP scheme.

Overall efficiency is:

$$\eta = \frac{p + q}{f} \tag{2}$$

where: η is an overall efficiency which is the ratio of all energy outputs to all energy inputs; p is an output of electrical energy; q is an output of useful heat; f is a fuel energy input.

If η is higher than 75% or 80% (depends on technology) than total electrical energy is an energy from cogeneration, if η is lower than we have to calculate a part of energy which is from cogeneration.

$$p_{CHP} = q_{CHP} \cdot C \tag{3}$$

where: p_{CHP} is an amount of electricity from cogeneration; C is a power to heat ratio.

Practical example:
p_{CHP} 20 units, q 65 units, f 100 units.

Overall efficiency is:

$$\eta = \frac{p+q}{f} = \frac{20+65}{100} = 0.85 = 85\% \tag{4}$$

Sensitivity of primaryenergy savings (PES) on useful heat shows Fig. 4.

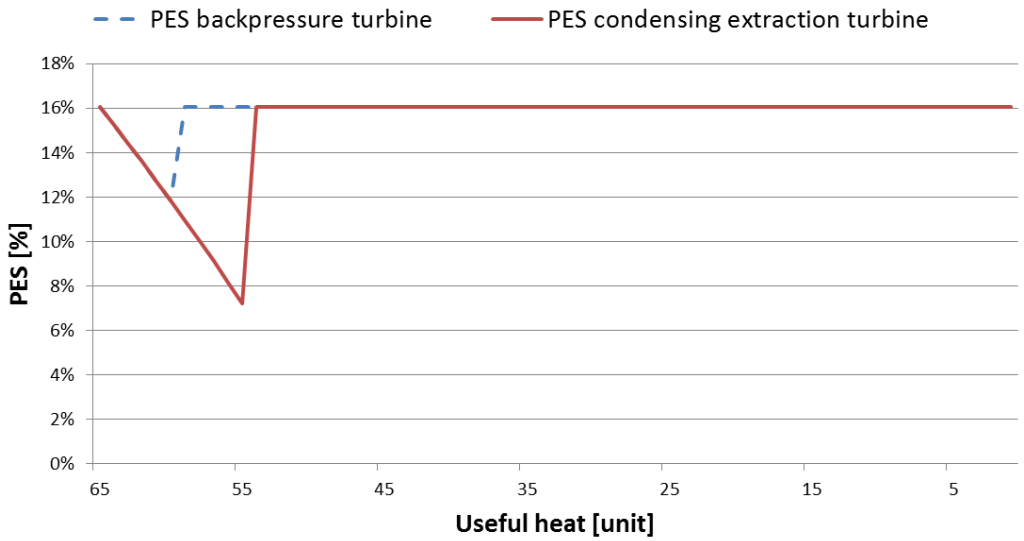

Figure 4. Sensitivity PES on useful heat, backpressure turbine and condensing extraction turbine.

The most importat information is in Table 1. Table shows a sensitivity of PES on useful heat. When an overall efficiency of condensing extraction turbine is between 75% to 78%;for a producer it is better to waste heat because a PES parameter is higher than 10% i.e. you are a high efficient cogeneration and you can receive a support from a government.

Table 1. Theoretical calculation backpressure turbine and condensing extraction turbine, primary energy savings and other important parameters

q_{CHP}	65	64	63	62	61	60	59	58	57	56	55	54
η	**85%**	**84%**	**83%**	**82%**	**81%**	**80%**	**79%**	**78%**	**77%**	**76%**	**75%**	**74%**
p_{CHP} backpressure	20	20	20	20	20	20	18.15	17.84	17.53	17.23	16.92	16.61
p_{CHP} condensing	20	20	20	20	20	20	20	20	20	20	20	16.61
p_{NOCHP} backpressure	0	0	0	0	0	0	1.85	2.15	2.46	2.77	3.08	3.38
p_{NOCHP} condensing	0	0	0	0	0	0	0	0	0	0	0	3.38
f_{NOCHP} backpressure	0	0	0	0	0	0	9.23	10.77	12.31	13.85	15.38	16.92
f_{NOCHP} condensing	0	0	0	0	0	0	0	0	0	0	0	16.92
f_{CHP} backpressure	100	100	100	100	100	100	90.76	89.23	87.69	86.15	84.61	83.07
f_{CHP} condensing	100	100	100	100	100	100	100	100	100	100	100	83.07
η_E backpressure	20%	20%	20%	20%	20%	20%	20%	20%	20%	20%	20%	20%
η_E condensing	20%	20%	20%	20%	20%	20%	20%	20%	20%	20%	20%	20%
η_T backpressure	65%	64%	63%	62%	61%	60%	65%	65%	65%	65%	65%	65%
η_T condensing	65%	64%	63%	62%	61%	60%	59%	58%	57%	56%	55%	65%
PES backpressure	16%	15%	14%	14%	13%	12%	16%	16%	16%	16%	16%	16%
PES condensing	16%	15%	14%	14%	13%	12%	11%	10%	9%	8%	7%	16%

Fig. 5 shows sensitivity of PES parameter on an overall efficiency, as you can see there is same situation like sensitivity on useful heat.

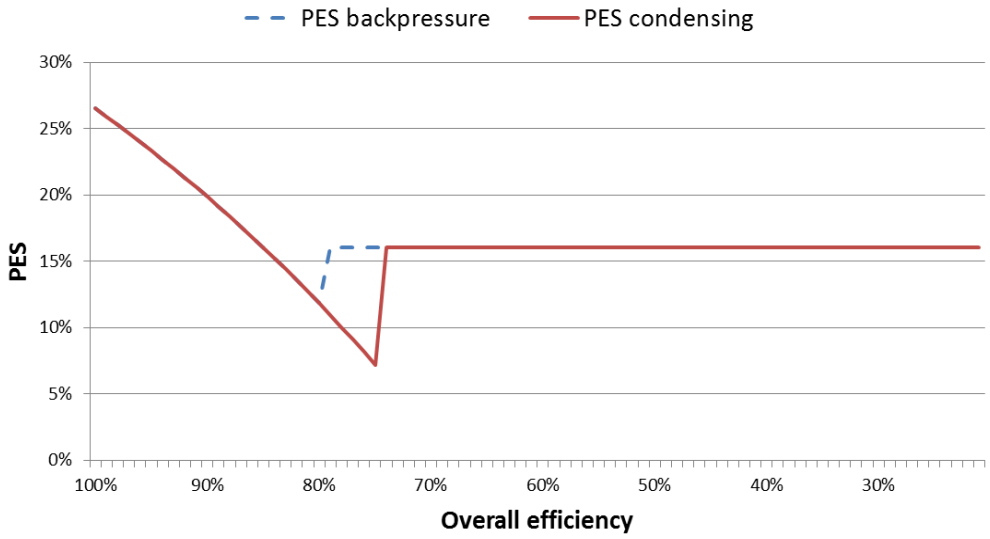

Figure 5. Sensitivity of PES on an overall efficiency, backpressure turbine and condensing extraction turbine.

Fig. 6 shows PES sensitivity on an overall efficiency with a different value of useful heat and cogeneration electricity.

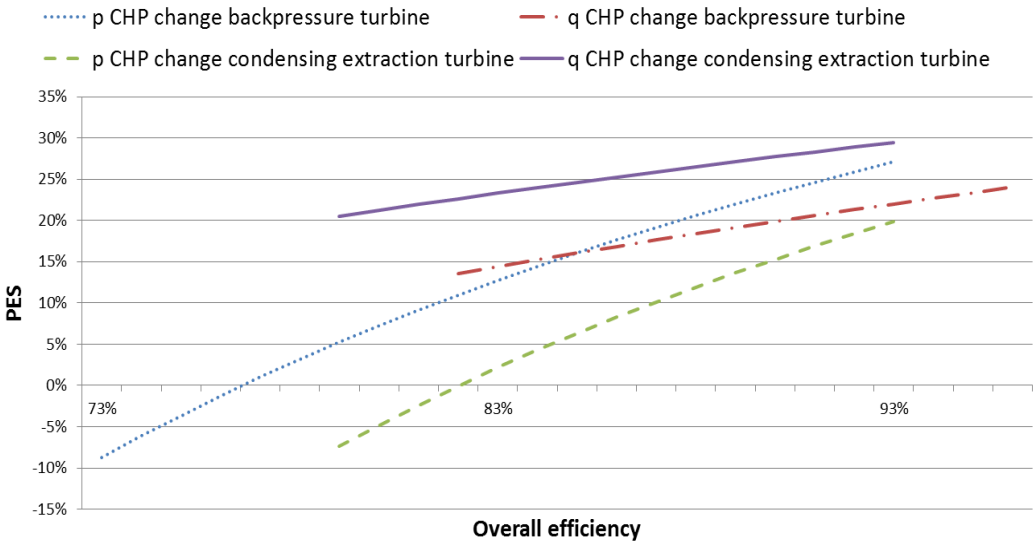

Figure 6. Sensitivity of PES on an overall efficiency, backpressure turbine and condensing extraction turbine.

Fig. 7 shows sensitivity of a PES parameter on an overall efficiency with a different value of electricity from a cogeneration. As you can see the most important parameter is electricity from a cogeneration, if this parameter is too low, then you have no chance, even you use all heat which you have, to get a better PES parameter. Negative PES parameter means that it is better to have a separate production of electricity and heat. For a better understanding the goal of a producer is to get a support.

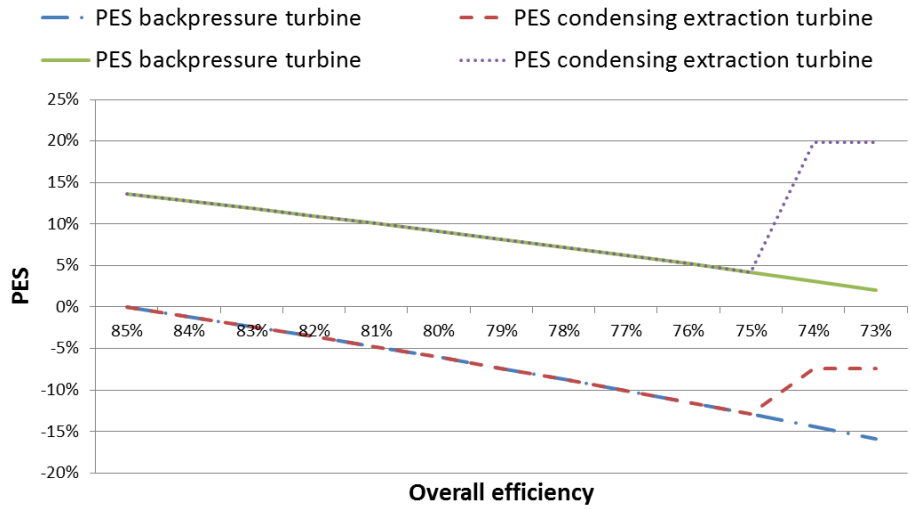

Figure 7. Sensitivity of PES on an overall efficiency, backpressure turbine and condensing extraction turbine with different values of electricity from cogeneration and different values of useful heat.

CONCLUSION

This paper briefly explains basic principles of a cogeneration andthe main legislation framework. The main focus is on primary energy savings parameter (PES) and sensitivity on heat waste. In a specific situation is better to waste heat according to use it. This is to be done because of virtual splitting possibility.

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The human factor's impact on the process of milking

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Abstract. This article explores the results of measurements that monitor human impact on the process of milking. Milk is an animal product that is intended for direct consumption but also for further processing. After obtaining milk from the mammary glands of dairy cows, it is treated so that it can be distributed to the customer in many forms. The customer may choose from a wide range of dairy products, which include milk as well as cream, cheese, yoghurt, etc. Large quantities of quality milk (from which other products are manufactured in consecutive steps) are used to satisfy the demand of consumers. The quantity and quality of cow milk is important for both the consumer and producer. This article discusses measurements focused on the effort of improving the preparation of cows before milking. This is the most important operation to ensure the best and fastest way of milking. A procedure for the preparation of the udder was proposed on the basis of professional literature and then measurements were performed in a building with a herringbone parlour. After evaluation it was revealed that the periods of milking were too long because insufficient preparation before milking. The performed measurements showed that when the udder had been prepared in the recommended way, the entire milking process showed significantly better results (shorter times of milking). The human factor can be therefore considered to be the milkers' behaviour towards the animals. It affects the welfare of dairy cows. We therefore recommend using the suggested process for preparing the udder.

Key words: herringbone parlour, milking equipment, mammary gland, teat cups, milking procedure, milking preparation.

INTRODUCTION

Milk is one of the most important components of food in our life. Humans and all other mammals world-wide feed on milk from their birth. All young mammals are fed by their mothers with milk (with colostrum at the beginning) from the mammary gland. However, milk accompanies humans also in other stages of life. Humans are the only adult mammals who consume the milk of other mammals (cow, goat, sheep, etc.). The most frequently consumed milk is cow milk. It contains easy-to-digest proteins, vitamins and minerals. It is almost an everyday part of our menus. The main way of obtaining milk is currently through milking machines in milking parlours (Gaworski & Leola, 2015). Machines do most of the work for people. They are constructed so that they copy the natural way young animals suckle and harm the adult animal to the smallest possible extent. Although these machines are already almost perfect, a lot of human work is still

needed for their servicing. The most significant task of a person performing machine milking is to ensure the necessary quiet conditions for the dairy cows and prepare them before milking. In terms of milk ejection, perfect udder stimulation before milking is necessary, as it leads to larger quantities of healthy milk being produced (Šimon et al., 2013; Rajaniemi et al., 2015).

The most important factors directly affecting the milking process include:

- Human impact–the milkers' behaviour towards the animals is always important, it affects the welfare of the dairy cows and the resulting performance. The milker should be friendly and calm and must not endanger the animal in any way.
- Udder stimulation impact–good stimulation contributes to faster and better milking, and it is therefore necessary to observe the correct procedure for udder preparation.
- Milking under-pressure impact–under-pressure should not be too high, as it can lead to teat damage. Too low under-pressure, on the other hand, leads to the lengthening of milking time, incomplete milking and subsequent decrease in performance. The average under-pressure should be around 32 kPa (50 kPa is the limit). The under-pressure should not fluctuate (Přikryl & Maloum, 2010; Přikryl et al., 2015).
- Pulsation impact and its ratio–the pulsation ratio has a positive impact on the duration of milking; with increasing pulsation the occurrence of mastitis decreases but the number of teat injuries increases (the optimal pulsation value is 60:40).
- Teat collar impact–these parts of milking machines, which are in direct contact with the dairy cow's teats, must be in perfect condition so that their defects would not disturb the milking process (for example, due to the set dropping, etc.) and their sizes must also be in correspondence with various teat sizes.
- Teat disinfection impact–using disinfection is an important step to prevent the occurrence of mastitis. Bacteria occur on teats and multiply if there is teat damage, which can lead to an infection. Disinfection immediately after milking causes the channel to close and the danger of transferring infection inside the teat is reduced.
- Impact of the time of milking in the parlour throughput–it represents the number of cows milked with one set of milking equipment per hour (Tančin et al., 2001).

The initial durations of milking measured were too long and above the recommended limit, which is why we investigated the possibilities of improving this process.

MATERIALS AND METHODS

Preparing the mammary gland before milking is the most important operation within the entire milking process. It has a hygienic, physiological and preventive function. The procedure focuses on tissues related to milk ejection. The main task is to stimulate the dairy cow's udder so that the greatest possible amount of the oxytocin hormone could be released and the milking could be as effective as possible. Oxytocin is released approximately one minute or one and a half minutes after the milker's first touch. This time limit must be observed and all necessary acts must be carried out within that time limit. Good preparation and stimulation of teats increases the pressure in the udder, the cow is milked in a better and faster way. The benefits of this are that the cow is in better health and the obtained milk has greater benefits. Preparation before milking

should be performed as follows (BouMatic, 2015): expressing the first milk; teat disinfection; teat cleaning and drying (stimulation).

All these operations must follow each other directly so that the time of stimulation by the milking machine fitting is maintained at a stable level (60–90s in the optimal case). Expressing the first milk means at least 6 control streams for each quarter. Thus most infected milk that could be produced between the individual milking sessions due to poor hygiene will be removed from the milk tank. Udder health and milk quality must be also checked. The colour and consistency of milk must be inspected. The first part of milk should be tested in a special control cup with a black bottom where any changes can be seen more clearly. Milk should never be expressed to the animal litter, onto the floor or hands of the milker. The first part of milk can help to discover possible inflammations in the udder (mastitis). In case of any suspected mastitis check streams must be performed repeatedly in special testing bowls with four holes into which a special chemical solution is added, which will acquire a different colour if the dairy cow has a disease. After the first part of milk has been milked the teats should be disinfected and subsequently cleaned and dried. This can be done in several ways: using the so-called 'wet toilette' is a less suitable tool and it is not very recommended, as it requires large amounts of water and the repeated use of a single towel, which deteriorates milk quality. The most suitable way known today is treating the teat with a disinfectant and cleaning and drying it with towels after that (Hulsen, 2011). In practice special jars are used for disinfectants (foaming non-returnable disinfectants) and the teats are soaked with the disinfectant located in the upper part of the jar. After repeated pressing the vessel forms foam (Pre-foam+ etc.) from a special solution, which must be spread on the teat and allowed to work for 30 s (or even better for 1 minute) because of the characteristics of the active substance. Thanks to the non-returnable design the vessel prevents the already used solution from flowing back to the flask. After that the teats must be cleaned and dried.

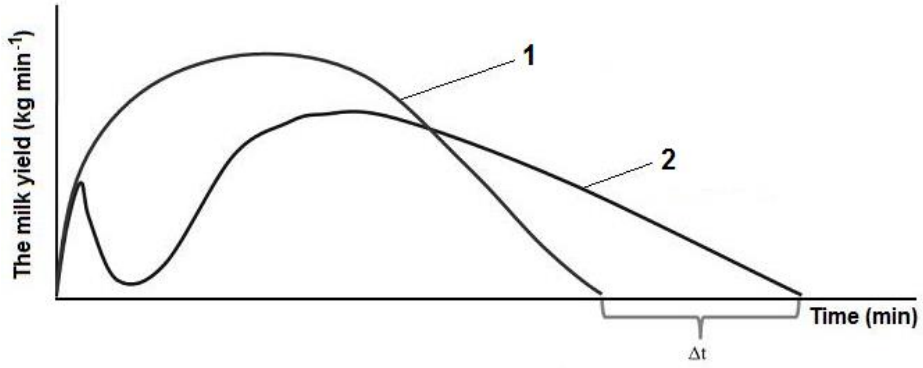
There are also several other options: either using textile towels soaked in disinfecting solutions, which are commonly used to clean the teats and duly washed and disinfected after use, or single-use paper towels, which are currently more frequently used due to their advantages, i.e., one towel is used per dairy cow and discarded afterwards. There is, however, also a third option, i.e., disinfection and cleaning in a single step by means of special towels soaked with a cleaning and disinfecting agent (Sani-WipesTM, etc.). Their advantage is that they make the milkers' work easier, help to prevent mastitis and can be easily recycled. After the first milk is expressed, the teats are treated with these towels and the milking machine can be fitted immediately. During cleaning the teats required stimulation is also performed. Each teat must be massaged with rotating downward directed moves for at least 4–6 s, which helps to stimulate and is also the proper way to clean a teat. Teat tips should be also massaged, as it is where most of the nerve endings can be found. This leads to the maximum filling of teats. The milking machine must be fitted immediately after that (BouMatic, 2015).

RESULTS AND DISCUSSION

In the chart (Fig. 1) it can be seen how the overall milking process should be performed with good preparation before milking, and in comparison, how milking proceeds when the preparation was poor. After having compared both curves, it can be

seen that if the preparations were good, the milking time is much shorter and the entire course of milking is ideal, i.e., the milk flow increases to the maximum (top flow) during the first minutes and afterwards the trend is decreasing. It shows that the oxytocin hormone is well released and it works ideally during the entire milking process. The dairy cow has been fully milked. To the contrary, if the preparation was poor, there are several peak points. The first peak point occurs when milk leaves the milk tank. After it empties, however, the milk flow decreases to zero as oxytocin has not been released yet. It is released after the teats have been stimulated by the teat collars (this can be repeated several times). This, however, leads to a period of dry milking (without milk), which prolongs the milking time and also the effect of under-pressure to the teats that are negatively affected.

Thanks to correct stimulation and observing the proper time for fitting the milking set we should obtain at least 50% of milk within the first two minutes and the overall milking time should not exceed 5 minutes, i.e., it is 15–20% faster than in cases when changes in stimulation occur. If this is achieved, we can be talk about the correct milking procedures leading to increased performance and the minimization of health problems in dairy cows.



Explanatory notes:
1 the curve of proper stimulation (preparation) of the udder (after changes)
2 the curve of poor stimulation (preparation) of the udder (before changes)

Figure 1. Comparison of milking curves with poor and good preparation (stimulation) of the udder (the curves show the final average values of fifty milking sessions).

Over-milking is the main cause of high somatic cell counts in milk. Approximately 0.1 L of milk should remain in each quarter of the mammary gland. So-called dry milking should be prevented. When the milking equipment is removed, the teats must be disinfected immediately. Timely disinfection helps the teat channel to close, which prevents the spread of bacteria. The regular disinfection of teats after every milking contributes to the good health of the udder and prevents mastitis. Measurement were made in a herringbone milking parlour with 2 times 6 milking positions. The milking procedure used in the parlour was as follows:

1. Expressing the first milk–check streaks are expressed after six cows have been led to one side of the parlour. 1 or 2 streaks are usually expressed on the floor below the dairy cows.

2. Disinfecting teats before milking–teat disinfection is performed with disinfectant vessels containing disinfectant agent (preparation 'Pre-foam+' effective in approximately 30 s). Milkers soak the teats in disinfectat before each milking.

3. Cleaning teats–milkers use wet textile towels (previously soaked in water and duly rinsed) for cleaning the udder (teats). During milking the towels are placed in plastic containers. After use the towels are washed using a common washing detergent and reused. A different towel is used for each cow.

4. Milking–after teat cleaning milkers fit the milking machine.

5. Disinfecting after milking–Prepost is used as disinfectant after milking.

The preparation is applied directly on the teat with a disinfecting vessel.The udder preparation is thus not ideal at all in view of current expertise. During the milking procedure measurements were made every second day in the period from October to November 2014 to ensure that milking was performed by the selected group of workers and only during afternoon shifts (9 measurements in total). The target group of this experiment was a group of dairy cows (the most productive) comprising 55 cows. The milk flows were recorded in certain time periods (at 0–15 s, 15–30 s and 30–60 s) and they include the maximum flow and milk flow during the first two minutes of milking. The measured values connected to milking recorded before the suggested changes were made have been provided in Table 1.

Table 1. Measured values connected to milking before suggested changes

Date	Average time per cow (min)	Flow 0–15 s (kg min^{-1})	Flow 15–30 s (kg min^{-1})	Flow 30–60 s (kg min^{-1})	Peak of flow (kg min^{-1})	Milk for the first 2 min (kg)	Milk for the first 2 minutes (%)
19.10.2014	6.3	0.4	1.9	1.7	2.2	5.3	35
21.10.2014	6.4	0.3	1.8	1.5	2.1	4.9	31
23.10.2014	6.5	0.1	1.3	1.3	1.9	4.6	30
25.10.2014	6.9	0.2	1.6	1.3	2.1	4.9	31
27.10.2014	6.2	0.3	2.4	2.3	2.6	5.3	36
29.10.2014	6.3	0.2	2.1	1.8	2.4	5.1	34
31.10.2014	6.5	0.1	1.9	1.6	2.1	4.8	31
2.11.2014	6.2	0.4	2.3	2.3	3.5	5.4	37
4.11.2014	6.3	0.4	2.4	2.2	3.1	5.4	31

The recommended average time in which milking should be terminated is maximally 5 minutes. All values obtained at measurement dates exceed 6 minutes (Fig. 2). These values were the first reason for changing the existing milking tactics. Milking time is very important for the dairy cow. Longer milking time creates significant health complications for the dairy cow's udder and it prolongs the shift of the milkers.

Other important indicators of udder preparation are the following three flow values that indicate milk flows for certain time periods: first at 0–15 s, second at 15–30 s and third at 30–60 s. These three values should have a rising trend during the entire milking time and should never fluctuate. Fluctuation would mean that milking is started after the poor stimulation of the udder and the oxytocin hormone does not fulfil its function in

full. This, however, is visible from the measured values. The following chart shows how the flows at measurement dates before introducing changes decreased during the first minute of milking (Fig. 3).

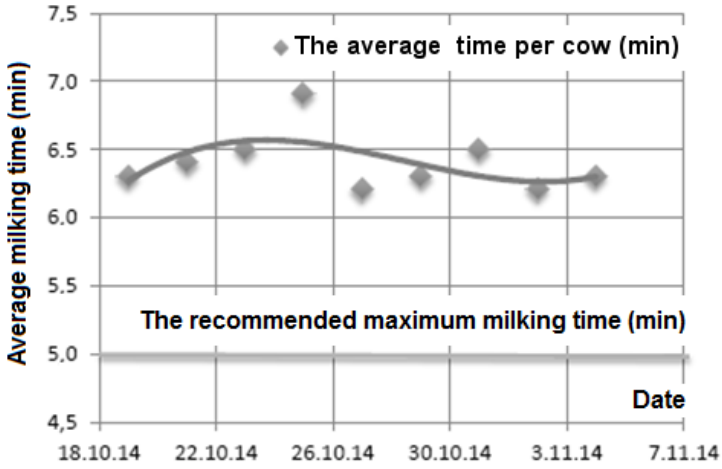


Figure 2. Chart showing the original milking times for the particular days before changes were made.

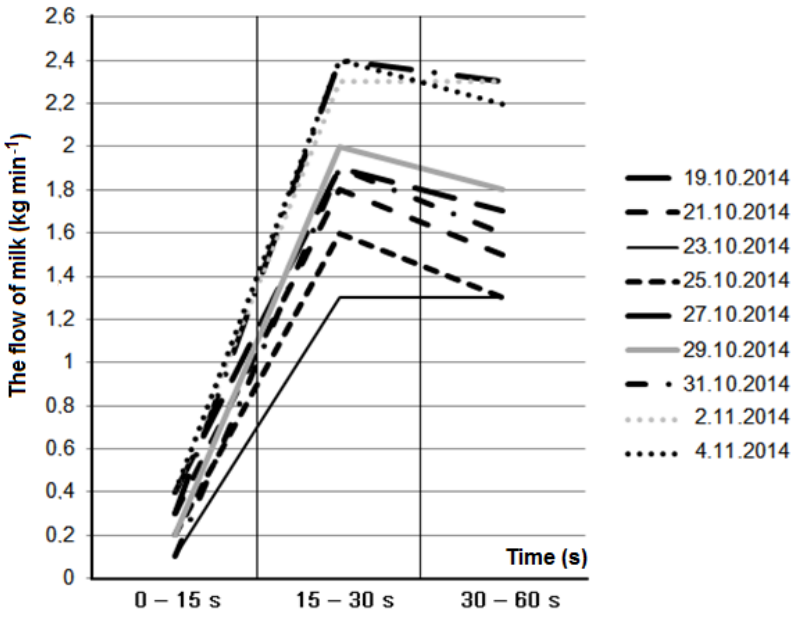


Figure 3. Original chart of flows at individual days before changes were made.

The decrease in the values recorded in the range of 30–60 s before changing the milking procedure compared to the values in the range of 15–30 s is significant. This decrease was most probably caused due to the fact that the oxytocin hormone was not

released as a result of poor udder stimulation, and the amount of milk flowing from the udders started to decrease. This is also the result of poor teat preparation before milking.

The last measured value is the amount of milk received during the first two minutes of milking. A duly stimulated cow udder should release 50% of the overall amount of milk during the first two minutes of milking. It can be seen from the last column of the measured values regarding the group milking outputs on particular measurement dates (expressed in %) that the amounts do not converge at 50% at all, i.e., the required milk output was not reached in either day. We can study the following chart (Fig. 4) for details.

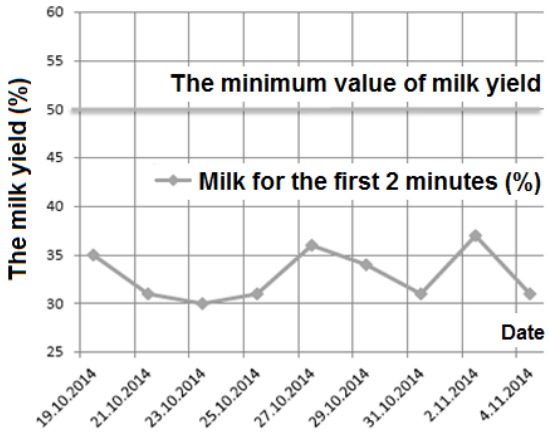


Figure 4. Daily milk outputs for the first 2 minutes before changes were introduced.

The results of this measurement therefore confirm the theoretical presumption that the poor preparation of the udder significantly affects overall milking performance. It can be seen from the originally measured values that this is the case in the milking parlour assessed herein.

Milking time has been exceeded by more than one minute, which means that the pulsating impact on the cow's udder could have been reduced by one minute. This can lead and leads to health problems.

The individual milk flows tend to fluctuate, which is typical for insufficient teat stimulation and failure to release oxytocin to the cow's blood. Udder stimulation reaches quite a low level and leads to poor milking and a significantly longer milking period. The discrepancies described above are the main reasons why this problem occurred. All these mistakes are mainly made by the milkers themselves and, therefore, this problem can be certainly rectified.

The following part includes basic recommendations to improve udder stimulation in the monitored milking parlour, which will lead to the overall better performance of dairy cows and the improvement of their health condition. The proposals have also been tested experimentally in this parlour. The recommended milking procedure involves all theoretical findings on correct milking (and is currently used in the monitored milking parlour). The procedure is as follows:

1. Expressing first streams–milkers always express 6 streams from each teat. The streams are caught in special cups.
2. Teat disinfection before milking–after expression and milk quality checks milkers perform teat disinfection before milking.
3. Teat cleaning and drying (udder stimulation)–milkers clean the teats with wet textile towels soaked in peracetic acid solution.
4. Milking procedure–after the udder is stimulated during cleaning the milking machine is fitted.
5. Disinfection after milking–when the milking machine is removed, milkers perform teat disinfection.
6. Milking machine disinfection–after each milking the milking machine is rinsed with peracetic acid solution.

The values measured during the recommended procedure are provided in Table 2. The table shows that milking time decreased after each measurement. Compared to the 6 minutes measured during the original measurement, the milking time was much shorter, i.e., less than 5 minutes, after training the milkers. Fig. 5 shows total time development.

Table 2. Measured daily milk outputs after the application of the recommended procedure

Date	Average time per cow (min)	Flow 0–15 s (kg min^{-1})	Flow 15–30 s (kg min^{-1})	Flow 30–60 s (kg min^{-1})	Peak of flow (kg min^{-1})	Milk, first 2 min (kg)	Milk for the first 2 min (%)
6.11.2014	6.0	0.5	2.5	2.7	3.6	5.7	38
11.11.2014	6.0	0.4	2.1	2.7	3.5	5.7	37
16.11.2014	5.4	0.7	2.5	2.7	3.0	5.4	40
21.11.2014	5.9	0.6	2.5	2.6	3.1	5.5	41
26.11.2014	5.9	1.0	2.4	2.7	3.1	5.3	40
1.12.2014	5.5	0.8	2.4	2.7	3.0	5.2	42
6.12.2014	5.6	0.7	2.4	2.8	3.0	5.2	40
11.12.2014	5.9	0.6	2.4	2.8	3.5	5.7	41
16.12.2014	5.3	0.8	2.4	2.7	3.1	5.2	41
21.12.2014	5.2	1.0	2.7	3.0	3.3	5.8	43
26.12.2014	5.8	0.7	2.5	2.8	3.6	5.6	40
31.12.2014	5.2	0.9	2.5	2.9	3.3	5.6	43
5.1.2015	4.9	0.8	2.4	2.8	3.2	5.4	51
10.1.2015	5.3	0.8	2.6	3.0	3.4	5.8	42
15.1.2015	4.9	0.8	2.4	2.6	3.3	5.4	49
20.1.2015	5.4	0.7	2.5	2.7	3.1	5.3	39
25.1.2015	5.4	0.8	2.3	2.8	3.2	5.4	44
30.1.2015	5.3	0.7	2.2	2.7	3.2	5.5	46
4.2.2015	5.4	0.8	2.1	2.7	3.1	5.6	49
9.2.2015	5.2	1.1	2.7	3.0	3.4	5.9	52
14.2.2015	5.3	0.9	2.4	2.9	3.6	5.8	51
19.2.2015	5.1	0.9	2.3	2.9	3.4	5.4	48
24.2.2015	5.0	1.1	2.5	3.1	3.6	5.6	52
1.3.2015	4.9	1.0	2.6	2.9	3.3	5.6	46
6.3.2015	4.8	1.0	2.3	2.7	3.2	5.3	48

The first sign of the fact that the milking procedure change has led to positive developments is the reduced milking time, which is nearly the maximum prescribed (5 minutes). Before the milkers' training this time was around 6 minutes. When the milking system and udder stimulation procedure were changed, the milking time started to shorten gradually.

Fig. 5 shows a declining curve displaying the average milking time from October 2014 to March 2015. It can be derived that improved teat preparation affects the length of milking. The shorter the time the better. Other indicators of the situation improving in the monitored milking parlour are the measured flow values for the particular measurement dates during the entire measurement period.

Fig. 5 fully corresponds with Table 2 (but the x axis does not show all measurement dates as in the table).

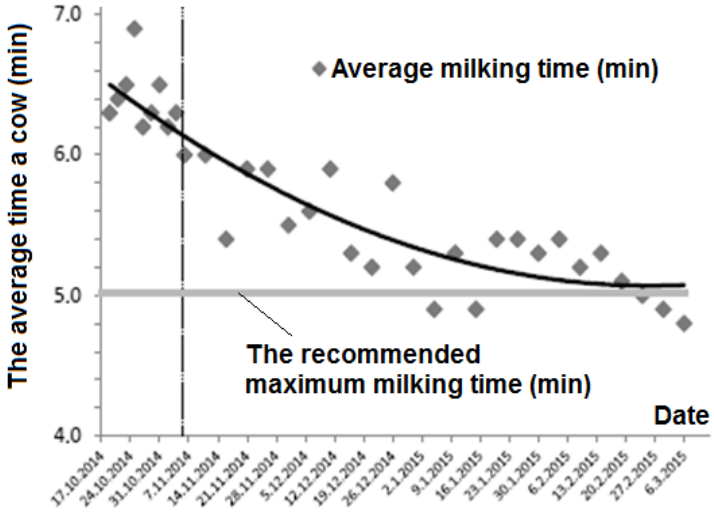


Figure 5. Chart of milking time development.

During all milking intervals the milk flows increased, while the flow values show an increasing trend (Fig. 6).

It means that the required situation has been reached in all milking sessions while the milk flow has gradually increased in the time period from 0 to 60 s. This confirms the fact that if the udder is well stimulated, oxytocin is duly released and great milk outputs are reached. In the first fifteen-second interval the milk flow ranged around 0.3 kg min^{-1}, by the end of this period it increased up to 1 kg min^{-1}. In the interval from 15 to 30 s the original flow amounted to approximately 1.9 kg min^{-1}, after the training it increased to the average amount of 2.4 kg min^{-1}. In the third interval of 30 to 60 s the milk output reached approx. 1.7 kg min^{-1} at the beginning, while at the end of the second measurement it amounted to up to 2.8 kg min^{-1}.

Compared to the measurements performed before the milkers' training, the new values indicated to an ideal situation and no measurements showed flow fluctuations. Owing to perfect stimulation the flows increased up to the top flow that also increased rapidly (Fig. 7) and then decreased very slowly until the milking machine was removed.

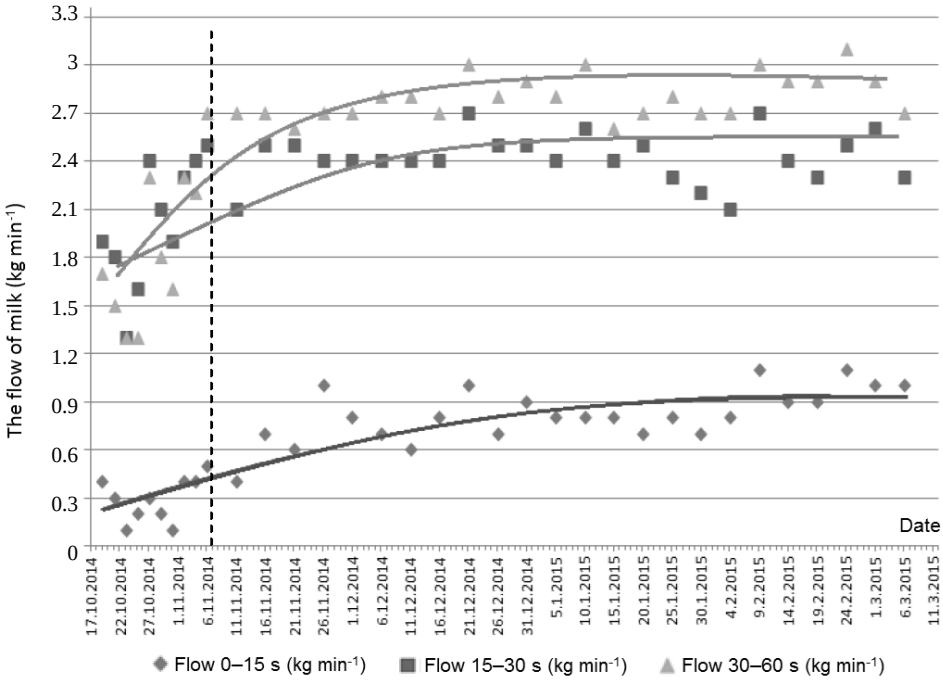


Figure 6. Chart of average flow development for the entire measurement period.

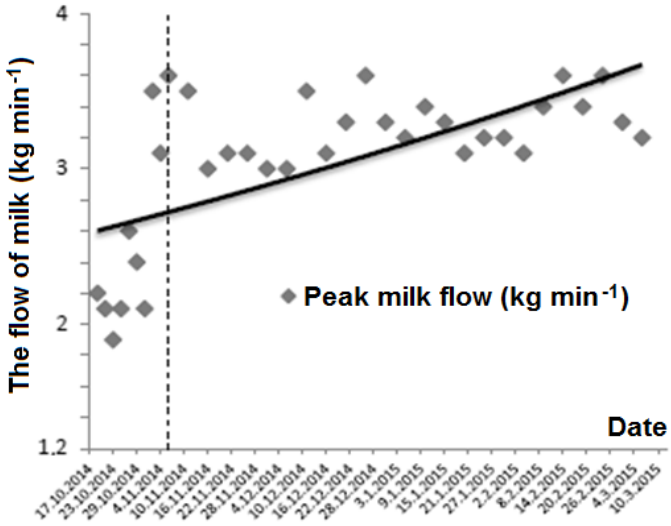


Figure 7. Chart of top flows in the period October 2014–March 2015.

The last value that can be compared in view of the previous measurements is milk output in % during the first two minutes (Fig. 8) of milking. During the first measurements the value was far below the limit, i.e., approximately 35%. After the milkers' training this value gradually increased until it finally reached the required 50%. This is another sign of the good stimulation of the udder before milking.

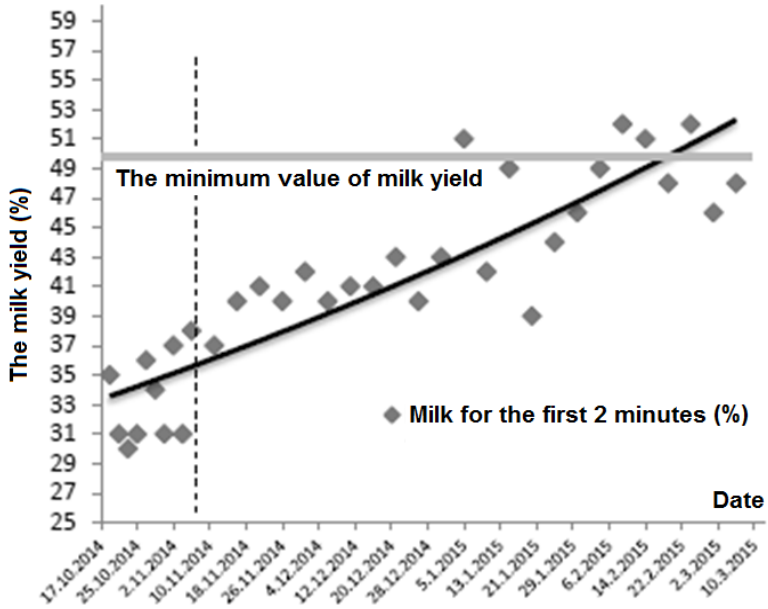


Figure 8. Chart of the milk output development during the first two minutes of milking.

CONCLUSIONS

The purpose of this contribution was to clarify the human impact on the milking process, and more specifically the ways in which people could affect dairy cow preparation before milking. Another task was to assess the most suitable preparation procedure and to recommend it for practical use.

On the basis of expertise on correct milking provided in literature, basic measurements were performed in the monitored milking parlour in the period from October 2014 to March 2015. The measurements were mainly concerned with the issue of milk ejection, which is undoubtedly the most important part of the entire milking process. To ensure fully-fledged ejection, proper udder stimulation must be performed before milking. A herringbone milking parlour with 2 times 6 milking positions was selected for making the measurements. Regular cow (mainly Holstein cattle) milking is performed in this milking parlour. The measured values were obtained with a computer programme. The programme records the overall milking times, flows in time, top flows and flows during the first two minutes of milking. These values clearly show how udder preparation affects the resulting milk output.

The first measurement was performed before changes were made to the milking procedure in the farm. The initial procedure contained five basic steps including wrong stimulation, which was not properly organized and resulted in the wrong performance and diseases of dairy cows. The individual steps were not performed in compliance with the expertise provided in professional literature. The values measured afterwards proved this. The time of each milking before the introduction of changes exceeded the 5-minute limit, which was recommended as the maximum value, by one minute. Flows in the particular time periods fluctuated, which clearly shows the poor stimulation of the udder, and the top flow values were also quite low (around 2.3 kg min^{-1}). The amount of milk

received during the first two minutes did not correspond to the required value of 50% (35% was measured). The measurements showed that the original procedure of udder preparation was entirely unsuitable.

The measurements were made in the same conditions after the introduction of changes, with the exception that before the second measurement the milkers underwent a special training course where they were introduced the proposal for the improvement of the udder preparation procedure before milking. This correct procedure that includes six basic steps was duly observed by the staff during the entire second measurement period. The output values were completely different from the values reached during the previous poor preparation. The milking times improved and fit within the 5-minute limit in most cases. The time flows gradually grew up to the average top flow of 3.2 kg min^{-1}, which is about one litre more than what was initially measured. The percentage of milk output also grew with each measurement and at the end it reached almost 50%.

The results of both measurements confirm that udder preparation before milking is a very important part of the entire milking process. It depends who and how performs the stimulation procedure. Dairy cows can be duly milked only if proper stimulation is performed. If a dairy cow is not ready for milking, it can create a number of complications both for the cow and milker. If we want to get milk with an increasingly higher quality, we should treat dairy cows increasingly better. We also claim that the proposed procedure observes all rules of proper milking and it can be recommended for practical use to get positive results.

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Economic considerations for using sexed semen on Holstein cows and heifers in Estonia

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Abstract. The study investigated economic and managerial considerations for using sexed semen as a tool for accelerated herd expansion and improvement of its genetic potential. Economic value of reproduction strategies based on conventional semen and sexed semen were analysed according to partial budgeting method by Victor E Cabrera and adjusted for the Estonian average indicators. Data for the study were provided by Animal Breeders Association of Estonia. In order to evaluate the economic value of using sexed semen over conventional semen, five different reproductive strategies involving sexed semen were used and compared with conventional semen-based strategy. Average conception rate from the first insemination with conventional semen was 65.6% and 56.1% with sexed semen for Holstein heifers in Estonia in 2015. Probability for birth of a female calf was 49.3% with conventional semen and 93.0% with sexed semen. Net present value for all sexed semen based reproduction strategies was negative at the baseline conditions. Sensitivity analysis for key reproductive and economic variables showed that market price of female calves and conception rates had the most impact on the economic value. Sexed semen can be a valuable tool for reproduction management in dairy farms, but the actual economic value of its application depends on the reproductive performance and objectives of an individual farm. Results of this study provide basis for further research about the situations, where using sexed semen would be economically justified for the farmers.

Key words: sexed semen, reproduction, conception rate, economic value.

INTRODUCTION

Although using sexed semen (SS) has gained popularity among Estonian dairy producers, there have been no studies proving the economic value of SS compared with using conventional semen (CS) on Estonian heifers. The article's methodology is based on Master's thesis by Jaak Härma (Jaak Naaber) (Naaber, 2014) and published in order to spread information from the thesis to improve management skills and support information-based decision making at dairy farms.

The objective of SS is to receive calves of the desired gender (Seidel, 2007; DeVries et al., 2008; Ghavi Hossein-Zadeh et al., 2011). This has created a possibility for dairy producers to accelerate improvement of genetic potential of the herds by getting more female calves from heifers that are genetically superior to the older cows (Seidel, 2007).

The sperms have to be divided into two fractions by the content of either X or Y chromosome to receive offspring of the desired gender (Jaakma et al., 2007). In order to receive a female calf, the cow or heifer has to be inseminated with sperm containing

chromosome X (Jaakma et al., 2007; DeVries et al., 2008). Due to time restrictions in the semen separation process, one dose of SS contains about 2 million spermatozoa that is approximately 10 times less than in one dose of conventional frozen bovine sperm (Olynk & Wolf, 2007; Seidel, 2007; DeVries, 2008). Resulting lower fertility of SS is therefore compensated with superior management and using SS predominantly on virgin heifers that have higher fertility than lactating cows (Seidel, 2007).

On average, the ratio of male and female calves born is 50:50 (Hasler, 2014; Jo et al., 2014). Using SS for insemination allows to determine gender of the calf by 85–95% probability (Fetrow et al., 2007; Seidel, 2007; Schenk et al., 2009; Butler & Wolf, 2010; DeVries, 2010; Hutchinson et al., 2013). As female calves become replacements for the herds, their births are important for the economic sustainability of the milk producers.

Economic value of using SS depends upon several criteria. The main benefit comes from higher probability for birth of female calves rather than male calves (Olynk & Wolf, 2007; DeVries, 2008; DeJarnette et al., 2009). Insemination with SS has a high probability of yielding the calf of the desired gender, but conception rate is lower with SS, compared with CS (DeJarnette et al., 2009; DeVries, 2010).

Studies on the return on investment of using SS have concentrated on heifers, as conception probabilities are lower for cows, even when using CS (DeJarnette et al., 2008). Considering that probability of conception decreases further with every unsuccessful insemination, it is economically viable to use SS only with the first insemination and with the following ones only if average conception rates for the whole herd are good (DeVries, 2008).

Several studies in the field of using SS have concluded that using SS in a dairy herd can provide the producers an economic profit compared with using CS, but it is different at each farm and depends on its reproductive and economic performance (Cabrera, 2009; DeVries, 2012; Olynk & Wolf, 2007).

Using SS enables dairy producers to expand their herds more efficiently compared with using conventional semen (Seidel, 2007; Hutchinson et al., 2013). Additional benefit lies in the possibility of internal herd expansion, i.e. without importing animals from outside the farm. It is important from both genetic and bio-security aspect, as introduction of externally sourced animals into herd can result in considerable increase in disease related problems (Faust et al., 2001). SS technology also allows for easier culling of less productive cows (Fetrow et al., 2007).

Objective of the study was to evaluate the potential economic value of insemination of Estonian Holstein heifers with SS as opposed to CS to help dairy producers make economically justified decisions about using SS in their herds. An additional objective was to test the possible advantage of using SS from herd reproduction aspect in the ideal conditions.

MATERIALS AND METHODS

Data for the study were supplied by the Estonian Animal Breeders Association (Eesti Tõuloomakasvatajate Ühistu, ETKÜ), whose 970 clients include the majority of Estonian dairy farms (Bulitko, 2016). ETKÜ supplied the data on reproductive performance of Estonian Holstein heifers and semen prices in 2013–2015. Overall usage of SS in Estonia is still low (Table 1). It should be noted that semen of some popular SS

bulls was sold by ETKÜ also as conventional variety; inseminations with these bulls were excluded from the study, as the records did not differentiate reliably between the varieties.

Table 1. Usage data of sexed semen on Holstein heifers in Estonia

	2013	2014	2015
Total inseminations	27,795	35,048	36,299
Inseminations with sexed semen (SS)	1,116	518	1,078
Share of sexed semen in total inseminations	4.0%	1.5%	3.0%
First inseminations with sexed semen (SS)	887	350	776
Share of first inseminations in total SS inseminations	79.5%	67.6%	72.0%

Source: ETKÜ.

Economic value

Methodology of partial budgeting of the survival curves (Cabrera model) (Cabrera, 2009) was applied on the data to evaluate the economic value of using SS over CS. Data received from ETKÜ was used to calculate the net present values (NPV) of various heifer reproduction strategies based on the Cabrera model using Microsoft Excel 2013 application. Using the Cabrera model, it is possible to evaluate the economic value of using SS compared with CS and test the expected additional income and expenses incurred by application of the new technology, assuming that all the other economic conditions remain constant (Cabrera, 2009).

In order to calculate the NPV-s of various heifer reproduction strategies, the basic formulas of the Cabrera model were adjusted to data about Estonian Holstein heifers that was supplied by ETKÜ. Use of NPV is justified because of the interval between consecutive inseminations. After five unsuccessful inseminations the heifer was culled and a pregnant heifer was bought as a replacement (Cabrera, 2009).

Five reproduction strategies using SS were constructed (SS strategy), based on how many inseminations would be done using SS, and compared with a strategy using CS (CS strategy) (Cabrera, 2009).

Sensitivity analysis was performed to evaluate the impact of the most important reproduction and economic parameters on the economic value of using SS versus CS. One or more parameters were changed in the Cabrera model for that purpose.

Reproduction indicators

Data from ETKÜ consist of the average reproduction and economic indicators from association members for Holstein heifers in 2013–2015; data from 2015 has been used as baseline data in this study. Reproduction indicators for heifers are subdivided into key indicators and supplementary indicators. Key reproduction indicators are related to the conception of heifers and probabilities of birth of male or female calves (Table 2). Supplementary indicators are related to data required to calculate the economic value of SS (Table 3). ETKÜ also provided data on individual reproductive performance of 14 farms that were the largest users of SS in 2015.

Table 2. Key reproduction indicators for heifers of member farms of ETKÜ

	2013	2014	2015
Conception rate using CS	66.0%	64.4%	65.9%
Conception rate using SS	44.5%	56.3%	56.1%
Probability of female calf birth using CS	49.3%	48.7%	48.8%
Probability of female calf birth using SS	93.0%	93.0%	93.0%
Probability of male calf birth using CS	50.7%	51.3%	51.2%
Probability of male calf birth using SS	7.0%	7.0%	7.0%

Source: ETKÜ.

Probability for male calf birth was found by subtracting the share of female calves from total number of calves born (100%).

Table 3. Supplementary reproduction indicators for Estonian Holstein heifers

Age of the first insemination (months)	14
No of unsuccessful inseminations before culling a heifer	5
Interval between inseminations (days)	21
Decrease of conception rate with every repeated insemination (%)	5

Source: ETKÜ.

A heifer was first inseminated at the age of 14 months and repeated for 4 additional times if first insemination was not successful. The heifer was culled if it failed to conceive after 5 inseminations. Interval between inseminations was 21 days (if a heifer was observed in oestrus after insemination, then the next insemination would commence after 21 days from the previous one). Probability of conception was reduced by 5% on average with every following insemination.

Economic indicators

There is a range of parameters that have to be considered, when evaluating the economic value of using SS compared with CS. Parameters used in the study are listed in Table 4.

Table 4. Average economic indicators for Estonian Holstein heifers

Cost of one dose of conventional semen (€ per dose)*	6.67
Cost of one dose of sexed semen (€ per dose)*	24.54
Cost of insemination procedure (€ per procedure)**	18.00
Market value of a new-born female calf (€ per head)**	100.00
Market value of a new-born male calf (€ per head)**	75.00
Cost of treatment of dystocia case (€)**	7.00
Rearing cost of an unsuccessfully inseminated heifer (€ per day)**	2.00
Live weight of a non-pregnant, culled heifer (kg)**	550.00
Salvage value of a culled heifer (€ per kg)**	1.20
Market value of a pregnant heifer (€ per head)*	1,300.00
Discount rate (%)***	3.33

Source: *ETKÜ statistics; **ETKÜ estimates; *** (Bank of Estonia 2016).

Average price of one dose of CS and SS sold by ETKÜ was €6.67 and €24.54 respectively in 2015. Cost of one insemination procedure was estimated at €18.00. Total cost of semen dose and insemination procedure corresponds to cost of semen dose as defined in Cabrera model (€24.67 for CS and €42.54 for SS, respectively).

Results of all inseminations and reproduction strategies were computed in present values, in order to obtain economically fair results (Naaber, 2014). Average short-term interest rate (3.33%) charged by credit institutions from non-financial borrowers in agriculture, forestry and fishing in Estonia in 2015 was used as the discount rate for present values (Bank of Estonia, 2016). The same average interest rate for 2013 was 3.24% (Bank of Estonia, 2016), the difference would have no significant impact on the results of the study.

Impact on reproductive performance

Illustrative timeline (Fig. 1) was created to describe the impact on using SS on the reproductive performance of a herd. It was based on 10 heifers that were inseminated with SS and CS in ideal conditions. Duration of the timeline was 6.5 years and the ideal conditions were the following:

1. Conception rate with both SS and CS was 100%;
2. Probability of female calf birth with SS was 90%;
3. Probability of male calf birth with SS was 10%;
4. Probability of female calf birth with CS was 50%;
5. Probability of male calf birth with CS was 50%;
6. Stillbirths, abortions, diseases and other causes of premature death were excluded;
7. Cows were culled after three lactations;
8. All born female calves were used as replacements to the herd.

The following parameters were used to construct the timeline:

1. Gestation length: 9 months or 275–282 days;
2. Calving interval: 418 days;
3. Length of lactation: 305 days;
4. Length of dry period: 67 days;
5. Average age of the first conception of a heifer: 14 months;
6. Average age of the first calving: 23 months
7. Cows were culled after three lactations.

Although lactation period and dry period preceding calving have lasted for 374 days or slightly over 12 months in total, they were linked with calving interval (14 months) on the timeline. Age of the first conception was linked to the age of the first insemination. As ideal conditions presumed conception rate of 100% using both CS and SS, then the age of the first calving was linked to age of the first insemination (14 months) and length of gestation (9 months). As a result, the cows on the timeline were 23 months old at the first calving. Average age of Estonian Holstein cows was 4 years and 6 months in 2013 (Jõudluskontrolli Keskus, 2014), therefore the cows were culled after three lactations in the model (Naaber, 2014).

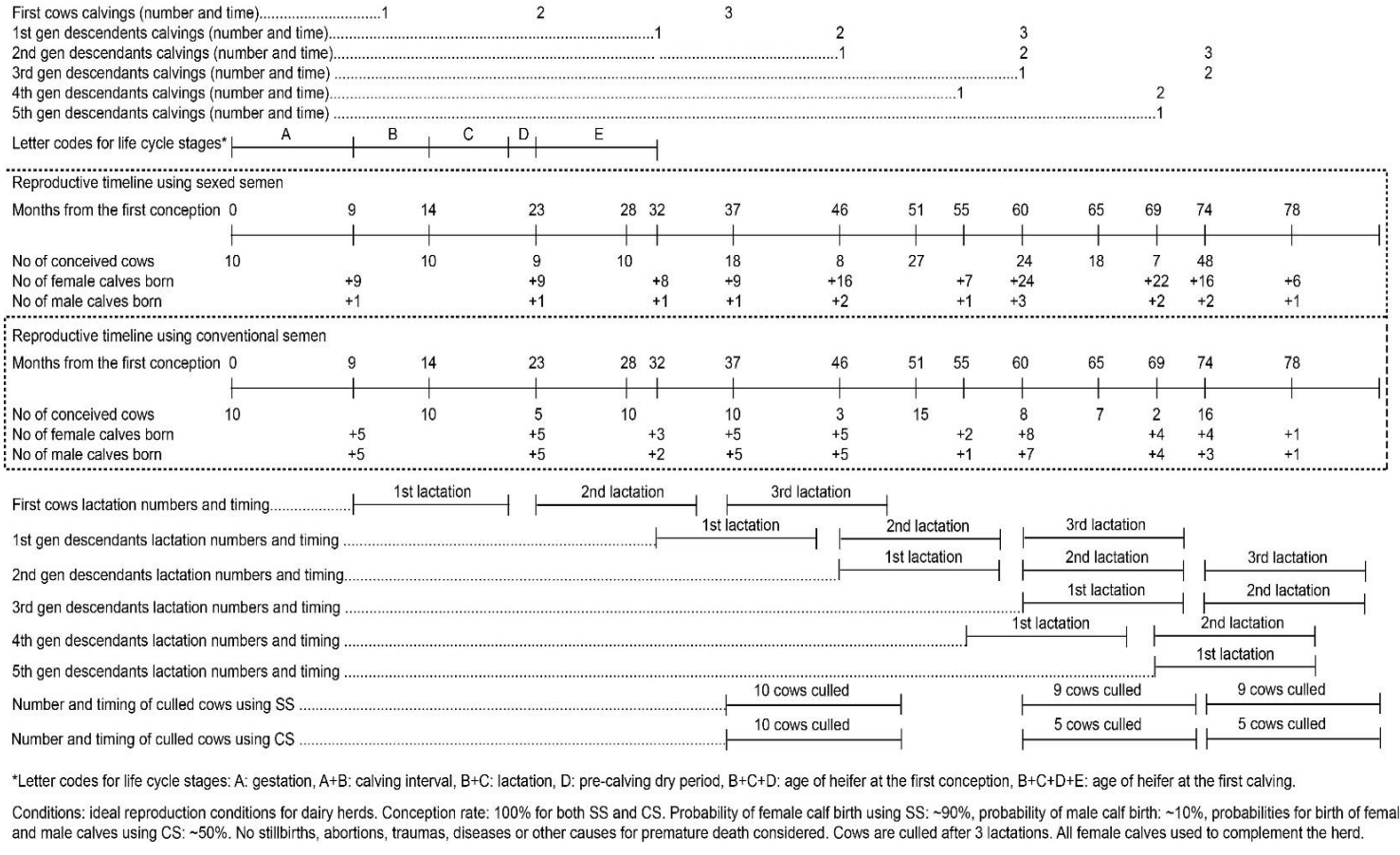

Figure 1. Reproduction timeline using SS and CS in ideal conditions. Source: Naaber, 2014. Updated with Estonian animal recording data from 2015, where applicable (Eesti Põllumajandusloomade Jõudluskontrolli AS 2016).

RESULTS

Economic impact

Results on the economic impact of using SS are presented in Table 5. For ETKÜ members, NPV of the first insemination of heifers with SS was -€24.43. Using SS for two of the first inseminations of heifers, NPV was -€47.30. Using SS for three of the first inseminations of heifers, NPV was -€69.35. If SS were used for all five inseminations of heifers, NPV would be -€112.44. This shows that at the given conditions, using SS is not economically profitable compared with using CS and every repeated insemination with SS increases the economic loss for the dairy producer compared with using CS.

Table 5. Economic impact of the reproductive strategies using sexed semen (2015 results)

First insemination with sexed semen	-24.43 €
2 first inseminations with sexed semen	-47.30 €
3 first inseminations with sexed semen	-69.35 €
4 first inseminations with sexed semen	-91.00 €
All 5 inseminations with sexed semen	-112.44 €

Source: ETKÜ.

Results of sensitivity analysis of the economic impact of using SS are presented in Table 6. Current analysis shows that if conception rate of the heifers using CS were 80%, NPV of the first insemination using SS strategy would decrease by €8.28. If SS were used for all five inseminations, NPV of this strategy would decrease by €27.40.

Decreasing conception rate of the heifers using SS by 10 percentage points, the respective economic loss from using SS versus CS would be €38.76 if using SS only for the first insemination. Compared with the baseline assumptions, the economic loss would increase by €14.34.

Raising market value of female calves by €200 per head, using SS for the first insemination would produce an economic profit of €23.60 over using CS. The results indicate that economic value of using SS depends the strongest on conception rate and market value of female calves.

Decreasing conception rate of the heifers inseminated with SS by 10 percentage points and increasing market value of a female calf by €200, the economic value of using SS or CS for the first insemination would be practically equal.

Overall low conception rates (50% for CS and 35% for SS) result in deeper economic loss from using SS that is not compensated for by higher female calf value.

If price of one dose of SS would decrease to €30 and market value of a female cow would rise by €100, then the economic profit of using SS for the first insemination were €11.88 With market value of a female calf at €300 and conception rates equal to the average of CS at 65.9%, using SS would produce an economic profit compared with CS in all strategies.

Table 6. Impact of reproductive and economic parameters on the economic value of using sexed semen technology at ETKÜ member farms

Conception rate using conventional semen (%)	Conception rate using sexed semen (%)	Cost of one dose of sexed semen (€)	Market price of a new-born female calf (€)	Economic value of using sexed semen (€)				
				Reproductive strategies involving use of sexed semen				
Baseline economic and reproductive parameters (Table 2,Table 4)				1	2	3	4	5
65.9	56.1	42.54	100	-24.43	-47.30	-69.35	-91.00	-112.44
Impact of one parameter on the reproductive strategies								
80	56.1	42.54	100	-32.71	-59.66	-85.12	-111.34	-139.84
65.9	46.1	42.54	100	-38.76	-73.42	-107.14	-141.76	-178.50
65.9	65.9	42.54	100	-10.38	-25.48	-41.85	-58.51	-75.17
65.9	56.1	30	100	-12.14	-22.84	-32.86	-42.59	-52.25
65.9	56.1	42.54	300	23.60	19.44	5.24	-12.99	-33.00
Combined impact of parameters on reproductive strategies								
65.9	46.1	42.54	300	0.02	-16.96	-42.58	-73.93	-110.18
65.9	56.1	30	200	11.88	10.52	4.43	-3.59	-12.53
50	35	42.54	100	-49.06	-95.36	-140.74	-186.31	-232.74
50	35	42.54	300	-21.11	-53.32	-92.01	-135.18	-181.97
65.9	65.9	42.54	300	46.71	49.41	39.37	25.23	9.67

Reproductive impact

Impact of using SS on herd reproduction was estimated by a comparative timeline of herd dynamics in ideal conditions using SS and CS. The first calves from heifers inseminated with both SS and CS in the ideal conditions were born during the 9th month of gestation. As there were initially 10 inseminated heifers in both groups, then there were 9 female calves and 1 male calf born from the SS group and 5 female calves and 5 male calves born in the CS group. The first cows calved on the 9th, 23rd and 37th months after the first conception and were culled from the herd after the third lactation. In total, the first cows from the SS group gave birth to 27 female calves and CS group to 15 female calves (Naaber, 2014).

As all female calves born were used to complement the herd, then the first generation of the offspring were inseminated on the 23rd month. First calves from the first generation offspring were born on 32nd month. The first generation of offspring gave birth to 8 female calves from SS group and 3 female calves from the CS group. The first offspring calved on 32nd, 46th and 60th month. In total for all three calvings, the first offspring from the SS group gave birth to 15 more female calves than CS group. Altogether, the first cows and their five generations of descendants gave birth to 126 female calves in the ideal conditions if inseminated with SS and 42 female calves if inseminated with CS during the observation period of 6.5 years. (Fig. 1)

According to the findings of the current study using SS can substantially increase supply of internally produced heifers compared with CS, enabling herd expansion, increase of genetic potential of the herd, or opening a new income stream from heifer sales, depending on the strategy of the farm. The economic analysis, in turn, suggested that, at the baseline conditions, choice of using SS does not create positive economic value for the dairy producers compared with using CS. Results of this study help dairy farmers evaluate the advantages and drawbacks of SS technology, considering their strategic objectives and the conditions at the farms.

DISCUSSION

Reproductive performance at the farm level

As conception rates are the highest from the first insemination (Kuhn et al., 2006), then performing only the first one with SS allows to realise a significant part of the potential reproductive benefit of SS, while limiting the economic loss per animal. According to the current study, the economic loss from using SS for the first insemination was €24.43, using SS for 5 (all) inseminations the economic loss would grow to €112.44 at the baseline conditions. The outcome is in line with other studies that have found that using SS for all inseminations would produce an economic profit only at practically unrealistic conception rates or very high prices of female calves (Olynk & Wolf, 2007; DeVries, 2012). Considering that, it was surprising that 20–30% of all inseminations with SS were repeated inseminations (Table 1). Moreover, on the aggregate level the number of repeated inseminations increased from 20.5% in 2013 to 28% of all SS inseminations in 2015, but at the same time, conception rate also increased significantly, from 44.5% to 56.1% (Table 2).

We hypothesised that these trends could be due to changes in the list of farms that used SS over the three-year period – farms achieving satisfactory results expanding SS use into repeated inseminations as well and those with poor results reducing or stopping use even on the first inseminations. Unfortunately, detailed data on individual reproductive performance in all farms using SS was not available, but ETKÜ could supply data on the 14 largest individual users of SS that in total used 52% of all SS doses sold by ETKÜ in Estonia in 2015. As some of these farms used SS mostly or exclusively on cows, data from 11 farms was analysed as part of this study to gain an insight into effectiveness of SS use on heifers at the farm level. Together, these farms performed 37.4% of all Holstein heifer inseminations using SS by the clients of ETKÜ in 2013 to 2015 (Table 2). Three years' data were analysed in aggregate form as annual datasets were relatively small.

Data suggests that intensity of SS use and conception rates do vary to a large degree and farms with the lowest results may be considering discontinuation of using SS technology, as based on the economic value analysis it is likely negative for them (Table 7). Based on this data, farms with below-average conception rates with CS tend to have uneconomically low conception rates with SS – confirming the view that application of SS in herds with conception problems is likely to deepen, not solve these problems. On the other hand, the data also suggests that it is possible to achieve high conception rates (over 55%) with SS. Managerial practices regarding application of SS technology at the successful farms warrant further research.

Table 7. Reproductive performance at selected farms using sexed semen on Holstein heifers in 2013–2015 (aggregate)

Farm no	No of SS inseminations	No of CS inseminations	Share of SS (%)	Conception rate (CR) using SS (%)	Conception rate (CR) using CS (%)	Difference in CR (%)
1	70	486	12.6	62.9	48.8	-14.1
2	236	2517	8.6	58.7	72.0	13.3
3	142	835	14.5	62.9	67.0	4.1
4	64	565	10.2	54.2	77.9	23.8
5	61	1,769	3.3	26.7	55.9	29.2
6	97	988	8.9	46.4	72.0	25.6
7	45	556	7.5	81.6	72.8	-8.8
8	65	99	39.6	53.2	54.2	1.0
9	107	363	22.8	44.4	58.4	14.0
10	84	891	8.6	35.1	53.9	18.8
11	44	351	11.1	58.1	74.0	16.0
Total	1,015	9,420	9.7	54.0	66.6	12.6

Source: ETKÜ.

Value of female calves

Sensitivity analysis demonstrated that value of a female calf has the strongest impact on the economic value of using SS. The result is in line with that of a broad-based feasibility study of factors affecting feasibility of SS (McCullock et al., 2013). A study of two large herds in the US recommended using SS for the first insemination to the herds that plan expansion. In their case, however, value of a new-born female calf was estimated at $250 and male calf at $50, meaning a fivefold difference in values (Chebel et al., 2010). De Vries (2008) refers to an even larger difference in values of male and female calves ($50 vs $450 per head)[1] (DeVries, 2008). In ETKÜ estimations, value of a female calf is only a third higher than that of a male calf (€100 and €75, respectively). There are also notable differences in the proportion of new-born female calf value to pregnant heifer value between suggestions of ETKÜ and data used in the US studies. De Vries (2008) estimates that sales price of a female calf is approximately 25% of the value of a pregnant heifer. Chebel et al. (2010) estimate that a female calf is worth 17.8% of the value of a pregnant heifer. ETKÜ data estimates that a female calf is only worth 7.7% of the value of a pregnant heifer. This gap implies that €100 per head value assigned to new-born female calves and thus the economic value of using SS may be underestimated. For a farm, intrinsic value of a new-born female calf is essentially the difference between market price of a pregnant heifer and cost of raising the calf into a pregnant heifer itself (DeVries, 2008). A comprehensive study of heifer rearing costs in Estonia is needed to confirm this hypothesis.

Impact of SS on stillbirths

Another aspect that needs further research is the impact of using SS on the incidence of stillbirths on the heifers. Results from the studies to date have been inconclusive. DeJarnette et al. (2009) found that SS technology increased incidence of

[1] Official exchange rate was 1.3917 USD/EUR as of 31/12/2008 and 1.0887 USD/EUR as of 31/12/2015 (European Central Bank, 2016).

stillbirths among the (unwanted) male calves, but it did not significantly affect the total incidence of stillbirths. Chebel et al. (2010) reported a significantly higher stillbirth incidence among female calves conceived using SS technology (10.7% vs 4.7% in one herd and 7.1% vs 3.8% in the other herd). Norman et al. found that incidence of stillbirths among single female calves was somewhat higher using SS than CS (9.7% vs 10.8%) based on 1.3 million inseminations in the US (Norman et al., 2010). No comparable data exists for Estonian herds today.

Herd expansion

Using SS also needs to be researched from herd expansion aspect. Average Estonian Holstein cow has the first calving at the age of 26.4 months and is culled from the herd at the age of 63 months (average for all dairy breeds) according to animal recording data (Eesti Põllumajandusloomade Jõudluskontrolli AS, 2016). With calving interval of 14 months, an average Holstein cow stays in the herd for 2.5 lactations. Accordingly, calves born to heifers represent approximately 40% of all calves born. If an average herd has also high calves and heifers culling rate, it is difficult to maintain the herd size without purchasing heifers from outside. SS could provide an alternative to heifer purchasing for such herds, while eliminating the bio-security risks related to outside animals.

CONCLUSION

Results from this study confirm that using SS enables dairy producers to increase supply of heifers to accelerate increase of the genetic potential and/or size of the herd from within the herd. However, the economic value of using this technology depends on the market prices of calves and reproductive performance of an individual herd. At the average reproductive performance at ETKÜ member farms in 2015 and respective market conditions, using SS is not economically justified.

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Performance trends for smart growth in the rural territories of Latvia

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Abstract. Any country is interested in economic growth regardless of its development level in the given period; yet an increasingly important role in defining growth is played by the term *smart growth.* The EU development strategy until 2020 defines smart growth as a strategic objective. For this reason, economic performance trends towards smart growth and smart specialisation have become an urgent task in project No.5.2.3 'Rural and Regional Development Processes and Opportunities in Latvia in the Context of Knowledge Economy' in National Research Programme 5.2. 'Economic Transformation, Smart Growth, Governance and Legal Framework for the State and Society for Sustainable Development – a New Approach to the Creation of a Sustainable Learning Community: EKOSOC-LV'.
The research performed by the authors gives an insight into the socio-economic performance trends towards smart growth in Latvia's regions and municipalities in particular, which are typical local administrative units in the country. The research used statistic data of the LURSOFT, Central Statistical Bureau and RDIM (Regional Development Indicator Module) databases for the period 2009–2013, examining the acquired information and performing horizontal and vertical analyses and data grouping, in order to identify the accumulation of positive/innovative changes. For a detailed examination of the mentioned phenomena, Zemgale region was selected as the territory in Latvia with an average development level. The research led to the conclusion that an increase of the proportion of knowledge-based goods (produced by high-tech and medium high-tech enterprises) and knowledge-intensive services in the overall increase of output in Zemgale region was greater than an increase in the total number of enterprises. The growth was observed both in 'accessible' territories and in 'remote' territories at different population decline rates etc. The latter allows considering that smart growth is determined not only by objective circumstances but also by local authorities, the activity of various public organisations and the readiness of residents to act under the guidance of the mentioned formations. It has to be taken into consideration when working on a territorial development strategy and achieving the objectives set in the strategy.

Key words: performance, smart growth, municipalities.

INTRODUCTION

In the Europe 2020 strategy, smart growth, sustainable growth and inclusive growth are defined as strategic objectives: smart growth is understood as developing an economy based on knowledge and innovation – promoting a more resource efficient, greener and more competitive economy and inclusive growth – fostering a high-employment economy delivering social and territorial cohesion (EC. Europe 2020). The

Europe 2020 strategy for smart, sustainable and inclusive growth can only be achieved if the territorial dimension of the strategy is taken into account, as the development opportunities of the different regions vary (EU, 2011). The place-based economic transformation role again highlights the EU National/regional innovation strategies for smart specialisation (EC, 2014a).

The present research is based on the methodology for rural analysis accepted in the European Union, which is known as 'the EDORA cube' and involves a three-dimensional framework for analysis – rurality/accessibility, a degree of economic restructuring and socio-economic performance, with a special focus on smart growth processes as the factors influencing economic performance trends (ESPON. EDORA...). The issue of smart growth in research studies refers to both a broad multidimensional approach and the observation of this phenomenon in a particular sphere of social life, for example, economics which outlines developing an economy based on knowledge and innovation (EC. EUROPE 2020). Smart and sustainable growth is not possible without radical innovations based on technological change that alter or replace traditional solutions. (EC, 2012) In practice this means to increase the share of a knowledge-based economy in the business as a whole.

Therefore the aim of the present study is to find out the knowledge-based economic growth in the rural areas of Latvia and conditions most significantly affecting the ongoing process by influencing agents as state institutions, local governments and communities in local territories.

In order to reach the aim, the following tasks were set:

1) to find out whether there is knowledge-based business expansion in Zemgale, one of Latvia regions, and if there is, then to what extent;

2) to evaluate agent groups' activities in business promotion or hindering.

The research on the issues regarding the economic growth in Latvia has been quite extensive (Vitola & Hermansons, 2012; Hilkevics & Stefenberga, 2014; Lonska, 2014; Garanti, 2015; Šipilova, 2015) yet, it is usually limited to the level of the regions which form the country. The authors' research for the first time provides an insight into the socio-economic performance trends towards smart growth in Latvia not only at a regional level but also at the level of municipalities, typical local administrative units in the country, thus contributing to the continuation of the 2009 administrative and territorial reform started in 2009.

MATERIALS AND METHODS

The research used Lursoft, Central Statistical Bureau and RDIM databases for the period 2009–2013, analysed the acquired economic information, performed horizontal and vertical analyses and data grouping. Zemgale statistical region, a territory with an average development level in Latvia, was selected for a more detailed examination (see Table 1).

Successful changes in the economic sphere occur when sufficiently large part of the residents perceive these changes positively and are ready to engage. Therefore simultaneously the survey of several social groups was carried out. Executive directors of Zemgale region's municipalities (n = 12) and social work performers (n = 39) as well as local residents (n = 103) were questioned to find out their opinions regarding the changes taking place in rural territories currently and the factors promoting or hindering

the changes in the economic sphere assessing the state institutions, local authorities and citizens in these changes.

Table 1. Internal disparities in Latvia at the level of regions

Region	Population density per sq. km in the beginning of 2015	Gross domestic product per capita (EUR) in current prices in 2013	Household disposable income per equivalent consumer in 2013, EUR
Pieriga	36	9305	579.96
Vidzeme	13	6944	448.72
Kurzeme	19	8497	490.85
Zemgale	**23**	**7152**	**432.93**
Latgale	19	6159	384.80

The results of the study could be taken into account when designing a future development scenario. The present research is closely related to the government-funded research project 'Rural and Regional Development Processes and Opportunities in the Context of Knowledge Economy' whose one of the key goals is the development of a strategy for smart rural and regional development (EKOSOC-LV 5.2.3.).

RESULTS AND DISCUSSION

I. Entrepreneurship trends in Zemgale region in the period 2009–2013

Even though the years of the global economic crisis were a complicated period, statistical data pointed to some increase in entrepreneurial activity. The total number of enterprises operating in the region increased and the number of enterprises performing innovative economic activity rose even faster. Consequently, the proportion of enterprises performing innovative activities increased as well (see Table 2).

Table 2. Increase in the proportion of HT, MHT and KIS enterprises (NACE Rev.2)

	Total enterprises in the region	HT, MHT and KIS enterprises	Proportion of HT, MHT and KIS enterprises
2009	2604	308	11.8%
2013	4215	612	14.5%
2013/2009	161.9%	198.7%	122.9%

* – The knowledge-based enterprises: MH – high technology; MHT – medium-high-technology; KIS – knowledge-intensive services.

Overall, the data of the region point to both the vertical growth trend and horizontal/structural changes, which are supported by the increase in the proportion of enterprises performing innovative economic activity. This means that, on the whole, the trend towards a knowledge based economy can be observed in the region. However, there is a question regarding whether the changes may be observed in all the municipalities of the region (20 municipalities), whether the changes have not only economic but also social influence, the most important among which is the maintenance of population.

The analysis of the data obtained from each municipality of the region has led to a conclusion that average figures may be used only as a criterion for comparative assessment of the situation in each municipality. In fact, quite different trends in entrepreneurial activity may be observed within the region.

First, although entrepreneurial activity has increased in all the municipalities of the region, the municipalities may be divided into two groups regarding an increase in the number of innovative enterprises (see Table 3). The first group consists of the municipalities where an increase in innovative entrepreneurial activity lagged behind the overall increase in business activity (this group comprises five municipalities), while the second group is made up of the municipalities in which an increase in the number of innovative enterprises was greater than an increase in the total number of enterprises (this group contains 15 municipalities). So, explicit structural changes took place during the course of development.

Second, the group of municipalities that presented a greater increase in innovative entrepreneurial activity also collected greater income tax revenues from enterprises operating in their territory. If the number of residents decreased in the municipalities, but the number of enterprises increased and the tax revenue paid by enterprises to the local governments increased in the analysed period, one has to assume that the smart growth process had taken place in all the region's municipalities. However, economic growth in the municipalities where an increase in innovative entrepreneurial activity was greater than the overall increase in business activity has achieved higher economic efficiency, which actually is the purpose of performance of a territorial unit (Stankevics, 2014).

Table 3. Percentage change in entrepreneurial activity for the groups of Zemgale region's municipalities in the period 2009–2013

Groups of municipalities	Total enterprises	HT, MHT and KIS enterprises	Increase in enterprise income tax revenue of municipalities
1st group of municipalities	168.3	129	122
2nd group of municipalities	168.6	225	133.9

Different growth paces led to structural changes in the distribution of enterprises. An increase in the number of economically active statistical units in such industries as agriculture and forestry (from 36.4% to 50.6%) was reported for the first group of municipalities, while growth, although insignificant (from 50.9% to 51.5%), was observed in the services sector for the second group of municipalities. Consequently, in 2013 in the region, there were municipalities that focused on economic activity in agriculture and forestry and there were also municipalities where a half of economic activity there was related to the services sector (see Table 4). However, a common feature is that manufacturing industry contributing to higher added value and labour productivity growth, played a quite small role in both groups of municipalities.

Table 4. Proportion of the dominant economic activities for the groups of municipalities in 2013

Groups of municipalities	Agriculture, forestry	Manufacturing	Services
1st group of municipalities	50.60%	9.95%	32.68%
2nd group of municipalities	25.15%	11.54%	51.54%

The indicators of some municipalities show also quite explicit internal disparities between both groups of municipalities. The proportion of manufacturing enterprises ranged from 2.1 to 14.8% for the first group and from 3.3 to 20.6% for the second group of municipalities. Since the municipalities with the highest proportion of manufacturing enterprises are border area municipalities and they are located outside the national and regional agglomerations, it could be assumed that rural areas refer to the space where growth of processing industry, associated with the group of high and medium high technologies, is possible similarly to cities.

The analysis of vertical economic growth and horizontal restructuring allows drawing the conclusion that smart growth as a performance trend in Zemgale region occurs, while regarding other regions of Latvia smart growth could be assumed to be hypothetical since the research and more detailed analysis of the data from other regions have not been completed yet.

II. Role of performance agents' groups in promotion of smart economic growth

According to the European Union's smart growth strategy, an increasing role in performance management is assigned to governmental institutions and community-led local development. Governmental institutions have to act as initiators and coordinators for the expansion of innovative activity in their administrative territories. This relates to both national institutions and local authorities that act as institutions administrating local territories. Good quality government institutions may be considered as an essential prerequisite for the development of effective innovation strategies (EC, 2014b). That is why it is of great importance that local governments do have the capacity for this kind of work. At the same time, the population of local territories are ready for such activity.

Community-led Local Development can mobilise and involve local communities and organisations to contribute to achieving Europe 2020 strategy goals of smart, sustainable and inclusive growth (EC, 2014c).

The dynamics of territory performance includes three phases:

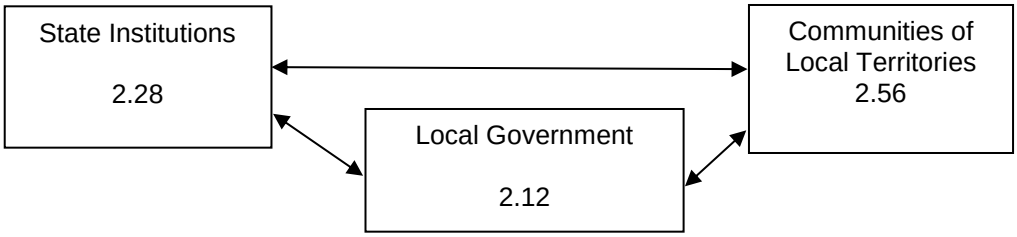
1) *Preconditions of performance* – agents have potential to achieve a certain level of performance in a municipality,

2) *Achievement of performance* – agents of a municipality reach a certain level of performance and become players in the national or global economic space,

3) *Result of performance* – to understand the municipality performance which has been achieved by the performance of agents as one of the factors that influence the performance (Stankevics, 2014).

However, transition from one stage to the next one within the country is affected by three groups of performance agents: the state as a set of institutions, local governments and communities of local people, which act as the Triple Helix model's elements that mutually interact (Ranga & Etzkowitz, 2013).

The authors were interested in the opinion among people regarding the influence of the agent groups on economic growth in municipalities, including knowledge-based growth, and in opportunities to determine which performance phase each agent group belongs to (see illustration in Fig. 1).



(Evaluation scale from 1 to 5, where 1 – very good, 2 – average, 3 – low, 4 – critical, 5 – no answer)

Figure 1. Evaluations of the groups of performance drivers by the respondents.

The results of the survey could be used for a more detailed analysis of the specific geographic place, but it was not the aim of this article. The authors were interested in the weighted average result of the agent groups and the most important factors promoting or hindering the necessary development.

The respondents considered local authorities to be the most important driver for change followed by state institutions, while the lowest evaluation was given to residents living in the administrative territory. However, the opinion of each respondent group brings some corrections in the performance evaluations of the agent groups.

- The executive directors of municipalities (who represented 60% of the total number of the region's municipalities) regarded state institutions, the kind of their activity and their performance as the most important agent group bringing change, whereas the role of local community members was rated very low.
- Social work performers ranked the authority of the local administrative territory in the first place and equally rated both state institutions and the local population.
- The municipality residents, assigning no priority to themselves, rated the authority of the local administrative territory in the first place and state institutions as performance drivers as the lowest (see Table 5).

Table 5. Evaluations of the groups of performance drivers by the respondents

Surveyed groups	Impact of		
	state institutions	local governments	communities of local territories
representatives from Zemgale region municipalities	1.83*	2.19*	2.87*
social work performers	2.40*	2.23*	2.40*
municipality residents	2.60*	2.15*	2.40*

* – Evaluation scale from 1 to 5, where 1 – very good, 2 – average, 3 – low, 4 – critical, 5 – no answer.

The information acquired allows identifying the most positive contribution of each agent group to rural development and the most negative activities or the lack of activity in achieving an objective from the perspective of the respondents.

The most positive result of **state institutions** regarding the development of rural territories, according to the respondents, was their efforts in making the EU funding available, but the following problems were mentioned as negative: the tax system that poorly contributed to or even hindered economic activity in the country and the operational principles of the financial equalisation fund.

The most positive specific activity of **local authorities** was their skill to attract the EU structural funding and to effectively use the funding, which was explained by the competence of the municipalities' officials in planning the development of their municipalities and in the management of development projects. However, the respondents mentioned the insufficiently integrated vision of the deputies elected in municipal government structures about the key objectives of territorial development and their insufficient work with local community groups to engage them in discussing and tackling important problems as the factors hindering their successful development.

The evaluations of **the local population's** contribution were different. On the one hand, the respondents stressed the local residents' readiness to acquire new and innovative knowledge, their readiness for economic activities aimed at raising their personal incomes and even their readiness for change in their job, community and surrounding environment. At the same time, the respondents pointed to the local residents' low readiness to actively cooperate in tackling practical problems in their municipality's life and their very low interest in economic cooperation. The respondents attributed high activity to 10.0–12.0% of the local residents and low activity or even reluctance to 35.0–45.0%. The results of this small survey are consistent with both international research studies on this problem (Keller, J.W.) and those done in Latvia (Paula, 2015) that reveal that the population cooperate mainly in the social, educational and cultural fields, while low or even insufficient activity is observed in the economic field in which the 'own government' (Pukis, 2010) has to not only make the population aware of state functions but also to play the roles of the community leader, initiator and promoter (Bariss, 2009).

The analysis of the opinions of respondents summarizes the directions of activities that performance agents have to primarily tackle with in order to favourably influence the pace of development and specifically the increase of the proportion of knowledge based entrepreneurship in the economic activities in the rural space. For the time being state institutions and local governments have mostly contributed their efforts in obtaining and using various EU funding, but they have not sufficiently explained the necessity of the change of the form and content of the economic sector according to new emerging requirements of the 21st century and have put limited efforts to enhance residents' inclusion into these changing processes. Therefore the sufficient pace of growth of successful innovative activities in entrepreneurship on the whole and specifically in knowledge-based entrepreneurship and faster growth of proportion of knowledge-based entrepreneurship have not been achieved yet which is proved by the analysis of LURSOFT un CSB data as well as by the results of the survey among the residents of the region.

The task of further research is, of course, to identify the most preferable fields of activity of municipalities and the most effective approaches to contributing to economic activity in rural areas in order to foster business, knowledge-based economic growth and the viability of rural areas under the modern complicated circumstances.

CONCLUSIONS AND PROPOSALS

Objective data indicate that even under the complicated circumstances caused by the global crisis economic growth trends could be observed in rural areas. The number of enterprises registered in the LURSOFT system in Zemgale region increased 1.6 times over five years. At the same time, the number of enterprises engaged in knowledge-based economic activity (HT, MHT, KIS) increased 1.9 times, which indicated not only quantitative but also qualitative changes. The proportion of knowledge-based economic activity increased. Unfortunately, the increase totalled only 2.7%-points over a five-year period, which makes us seek solutions to raise the pace of increase in the proportion of knowledge-based growth and in the proportion of the number of related enterprises in the total enterprises.

An analysis of quantitative knowledge-based growth reveals that the pace of growth differs among the municipalities. Based on the comparison according to two indicators (increase in the total number of enterprises and the number of knowledge-based enterprises) for the period 2009–2013, Zemgale region's municipalities may be divided into two different groups. In Group 1 (five municipalities), the total number of enterprises rose faster than that of knowledge-based enterprises; in the result, the proportion of knowledge-based enterprises decreased from 8.2 to 6.4% in those municipalities. In Group 2 (15 municipalities), an increase in the number of knowledge-based enterprises considerably exceeded an increase in the total number of enterprises, and their proportion rose from 12.1 to 15.0%. In two municipalities of the region, the proportion of this kind of enterprises was even greater than 20% of the total enterprises. Since the municipalities of Group 2 have both 'accessible' territories and 'remote' territories, an opinion forms that smart growth is not determined only by objective circumstances but also by subjective factors such as municipal government performance, the activity of various public institutions and residents' readiness to work under the guidance of the institutions and organisations.

The information acquired in the survey of executive directors of Zemgale region's municipalities, social work performers and residents give some insight into the perspectives of the mentioned rural community groups about the roles of performance groups on the way towards a smart economy. The respondents considered their municipal government to be the most important driver; yet they suggested it has to better play the role of the 'own government', expanding cooperation with the local population and the municipality's entrepreneurs. The respondents mainly perceived state institutions as those conducting the EU financial flows to municipalities, criticising instability in economic policies (tax policy and legislation). The third driver of performance is the community of residents themselves, as progress depends on their readiness for actions. According to research studies on the implementation of innovations in the real life, it takes time to get from the birth of an innovative idea to the 'integration' of the innovation into the society. The survey data show that Latvia's rural society is still at this stage, as only 10–15% of the residents actively engage in the

processes of change taking place in rural areas, which also influences knowledge-based economic growth.

The findings on the performance trends in Zemgale region of the present research make it necessary to verify the conclusions based on the data of other regions in order to contribute to the elaboration of Latvia's rural smart growth strategy in the context of knowledge-based economy. Secondly, discussions should be carried out on larger scale among all three groups of performance agents in order to find the solution to the problem.

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Choosing and evaluation of milking parlours for dairy farms in Estonia

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Abstract. The aim of this paper is to present the main criteria, which could be used for the choosing, optimization and evaluation of a milking parlour in two large capacity Estonian dairy farms. The choosing and evaluation of milking parlours parameters is based on the available information and results of previous research in dairy farms in the Estonia, using the mathematical model created in the Czech Republic. Time for milking and final specific direct costs are main parameters which enable evaluation and choosing of suitable milking parlour for the dairy farm. Calculation of the first farm with a capacity of 300 cows showed that in the case of rotary milking parlour with 32 milking stalls total specific direct costs per milking per cow and year would be by 25% higher than in the case of Side by Side milking parlour 2 x 12, but the time for milking would be reduced by about 25%. The second farm with capacity of 1,850 cows is equipped with a rotary milking parlour with 70 milking stalls. There are three milkers. Six milkers would bring shortening of one milking from 6.3 h to 3.3 h while preserving approximately the same total specific direct costs per milking per cow and per year. This milking parlour could be used also for the planned increase in capacity at farm to 3,300 cows. Time of one milking would be 5.6 hours, but total specific direct costs per milking per cow and per year would be reduced by 18%.

Key words: Costs, cows, equipment, farm, milking process.

INTRODUCTION

Livestock production in countries with intensive agriculture is undergoing big and rapid changes. Capacity of farms are expanding and increasing the average annual milk production per cow. These factors lead to modernization of milking equipment. European housing systems are steadily changing from stanchion barns towards loose cowsheds and larger herd sizes (Maton et al., 1985; Bottema, 1992; Hansen, 1999; Gaworski et al., 2013; Gaworski & Leola, 2014; Gaworski & Priekulis, 2014). Due to these changes, many dairy farmers will have to design and build new milking parlour systems.

The milking process is the key operation on dairy farms. The function of milking parlour is one of the factors which affect the efficiency of milk production on the farm. There are many problems which influence the choosing and proper use of milking

parlour. Some of them should be solved in advance during the preparation and design of dairy farm.

Modern large-scale farms require appropriate modern technical equipment. Equipment producers want to sell you the most expensive product which is not always appropriate. Operation is affected e.g. by selected number of milking stalls, by high or low number of milkers, sometimes by incorrectly selected or by choosing insufficient automation equipment. Therefore, it is important to compare different possibilities of milking parlours and try to find the strengths and weaknesses of some proposals. Model calculations allow comparing options and making decision according to the accurate and uniform criteria correctly according to the results of calculations.

Therefore it is very important to find the appropriate criteria that would allow choosing the optimal type of milking parlour, corresponding to the overall concept of the farm and meeting all operational requirements under acceptable economic conditions. The aim of this paper is to present the main criteria, which could be used for solution of principal questions important for optimization of milking parlour: technical parameters, indicators of labour productivity, and economic criteria.

The same milking parlours have different operating conditions in different countries around the world. Dairy farms in Estonia are interesting, because at present arise in addition to traditional small farms also new large-scale farms with thousands of cows. For these farms it is necessary to calculate (model in advance) different variants of equipment and operating conditions by precisely selected and uniform criteria.

MATERIALS AND METHODS

There are available solutions offered by manufacturers of either milking parlours, or automated milking systems (AMS), equipped with milking robots. Many books, reports and scientific publications present results of research and recommendations focused on the problems of AMS, usually also including comparison of AMS and milking parlours, in some publications information related to problems of performance and economic analysis e.g. (Bottema, 1992; Kic & Nehasilova, 1997; Kic, 1998; Priekulis & Laurs, 2012).

Leading companies producing milking equipment usually offer a variety of constructions of milking parlours recommended for different capacity of farms. They also recommend the possible level of automation and number of milkers which should work in the milking process (Brunsch, et al., 1996; Dolezal et al., 2000; Chiumenti, 2004). But there are rather big differences in local conditions of the farms according to the production, economic, market and labour situation of the country or province. Although the use of AMS for large farms with a big capacity is developing, the high cost of this solution discourages many farmers. The question for medium and large farms is to currently choose an appropriate type of milking parlour.

It is possible to say that there are two divergent interests and goals in choosing the appropriate type of milking parlour. On the one hand there is interest of manufacturer and dealer who strives for the highest price contract and on the other hand, a farmer who would like to receive the best parlour, but for the price as favourable as possible, i.e. the lowest.

There are various practical recommendations in the literature, however, there are usually not sub-economic data included which results in a specific numerical data,

characterizing the overall result of milking parlour solutions. Some publications (Provolo, 1992; Provolo & Marcon, 1993) present models focused on the choosing of milking parlours, but not in a complete universal approach which could be adapted everywhere. Results of research and basic equations used for calculation of several parameters of milking parlours presented by Gaworski & Priekulis, 2014. Similar calculations, completed with several important economic results which are valid for rotary milking parlours are presented by Ozolins et al., 2012.

Currently there are a variety of mathematical models, which can help us to optimize the solution of various functional dependencies. It is always necessary to find appropriate criteria for the decision-making process. Some results of optimization and calculation based on mathematical model focused on the conditions of dairy farms and milking production in Czech Republic are presented by Kic 2015a; and by Kic, 2015b. The following calculations are based on the same calculation model, just changed with parameters according to the data valid for the Estonian dairy farm and production conditions.

The question is which criteria would be suitable to determine the type of milking parlour for each farm. If we know them, according to them can be evaluated different milking parlours, as well as we follow them when consider specific aspects and individual issues which influence the selection of milking parlour for the farm.

For objective assessment and selection of milking parlours can be used and considered a lot of different aspects, e.g.: animal welfare, capacity, price, the number of milkers, the complexity and sophistication of the operation, reliability, the dimensions and complicated installation in the building, demand of maintenance and service, some other aspects.

Overestimating or underestimating some aspects may result in problems during the normal operation of the milking parlour in practice and thus negatively affect the operation of the farm. In some cases this may lead to unnecessary wastage of finance for investment, without any real benefit to the operation of the farm.

The first criterion which is important for the function of the farm is the time for milking. The fast milking of all cows enables to have enough free time in which cows have the opportunity to eat and relax, to go to pasture and so on. The duration of one real milking of all cows can be calculated according to the equation (1).

$$T_{vd} = \frac{N}{Q_{LS}} + T_{pr} \quad (1)$$

where: T_{vd} – the duration of one real milking, min; N – the number of lactating cows on the farm, cow; Q_{LS} – the real capacity of a milking parlour, cow min^{-1}; T_{pr} – the time of working breaks, min.

As regards of a human working process and working operations there is important the total time of duration of one milking including preparatory operations and finishing work after milking, calculated according to the equation (2).

$$T_{cd} = T_{vd} + T_p + T_c \quad (2)$$

where: T_{cd} – the total time of duration of one milking including preparatory operations and finishing work after milking, min; T_p – the time of preparatory work before milking, min; T_c – the time of finishing and cleaning work after milking, min.

When this period T_{cd} is short enough then there is enough time for workers (milkers) to carry out the other activities (feed preparation, cleaning, control of animals etc.). Therefore the time should be a criterion for optimization and the selection of a suitable milking parlour for the farm.

The second decisive criterion for choosing the appropriate milking parlour should be the economic criteria. It is necessary to compare the specific data, which are in this case the final specific direct costs of a milking parlour per cow and year $^{u}C_{MP}$, which are calculated according to the equation (3) as a sum of specific labour costs of milking per cow and year $^{u}C_{W}$, specific costs of the milking equipment per cow and year $^{u}C_{P}$ including the parlour construction, and specific costs $^{u}C_{S}$ of consumed supplies including the water, electricity, disinfections etc. per one cow and year.

$$^{u}C_{MP} = {}^{u}C_{W} + {}^{u}C_{P} + {}^{u}C_{S} \quad (3)$$

where: $^{u}C_{MP}$ – the final specific direct costs of milking parlour, EUR cow^{-1} year^{-1}; $^{u}C_{W}$ – the specific labour costs per cow and year, EUR cow^{-1} year^{-1}; $^{u}C_{P}$ – the specific costs of the milking equipment, EUR cow^{-1} year^{-1}; $^{u}C_{S}$ – the specific costs of consumed supplies, EUR cow^{-1} year^{-1}.

Specific labour costs $^{u}C_{W}$ are calculated on the basis of labour requirements per cow per year and average hourly wage of the milker.

Specific costs of the milking equipment $^{u}C_{P}$ are calculated as specific data of total operating costs of the milking machine converted per one cow. Therefore it includes the amortization of machinery, which is the purchase price of the machine expressed by percentage of machine amortization, further amortization of construction that includes construction costs and percentage of building amortization and the cost of servicing, maintenance and repairs, which are usually expressed as a percentage of planned acquisition costs.

Specific costs of consumed supplies $^{u}C_{S}$ are calculated as a sum of costs of all necessary operating materials and energy. The consumption of electricity is proportional to the power inputs of motors and all electrical appliances of milking parlour during their operation, water, disinfection etc. All is re-calculated per cow and year (EUR cow^{-1} year^{-1}).

Described criteria were used for evaluation of milking process in two types of farms typical for current situation in the Estonian agriculture. All data used for the calculation were based on the data from dairy farms in the Estonia. The first farm A is representing the medium dairy farm with 300 cows. There are calculated criteria and compared results between the variant A1 equipped with a milking parlour Side by Side 2 × 12 milking stalls and variant A2 equipped with a rotary milking parlour with 32 milking stalls.

The second farm B represents very special large scale dairy farm with 1.850 cows. There are calculated criteria and compared results between the variant B equipped with a rotary milking parlour with 70 parallel milking stalls. There can be used milking parlour with different number of milkers (variants B1 and B2). As the farm capacity could be increased to 3,300 cows is the variant B3 solved for the same milking parlour functioning in the conditions of this farm with 3,300 cows.

RESULTS AND DISCUSSION

The results of calculations of the farm A are presented on the Figs 1 and 2. Two milkers are supposed to work in both variants of milking parlours. There is a standard level of technical equipment in both variants of milking parlours. The variant A1 milking parlour Side by Side 2 × 12 milking stalls has lower labour productivity as well as lower milking capacity therefore the labour requirements are higher in this variant and time of one milking (Fig. 1) is longer than in the variant A2 (rotary milking parlour). On the other side milking parlour Side by Side is cheaper ($^{u}C_{P}$), which results in the lower final specific direct costs of milking parlour $^{u}C_{MP}$ (Fig. 2). The price of milking parlour A2 is higher because of the higher number of milking stalls, more complicated and sophisticated construction which results just in the bigger specific costs of the milking equipment $^{u}C_{P}$.

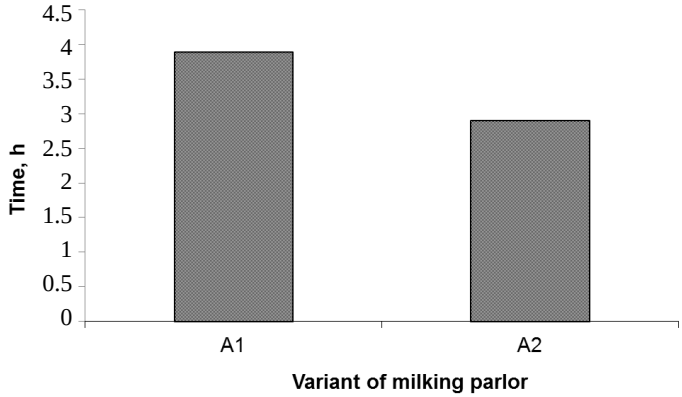

Figure 1. Time of one milking in the milking parlour A1 (Side by Side 2 × 12) and in the rotary milking parlour A2 (with 32 milking stalls) at the dairy farm A (300 cows).

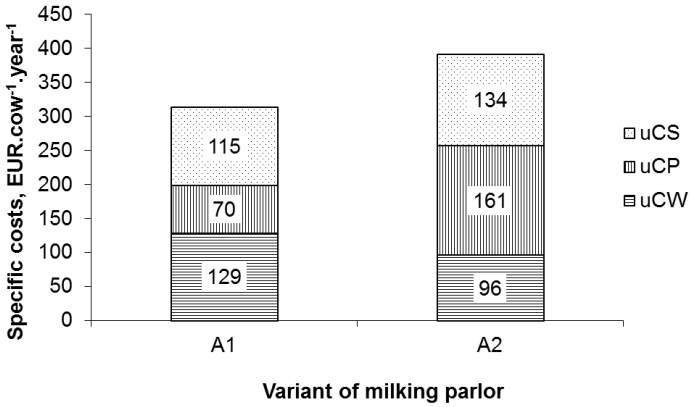

Figure 2. Specific costs of milking in the milking parlour A1 (Side by Side 2 × 12) and in the rotary milking parlour A2 (with 32 milking stalls) at the dairy farm A (300 cows).

The final decision of choosing the suitable milking parlour will depend on the priorities of the farmer, if he prefers cheaper variant solution A1 or more expensive variant A2 but with higher capacity and shorter time of milking.

The results of calculations of the farm B with 1,850 cows are presented on the Figs 3 and 4. These results of calculation are rather influenced by the real milking process as well as by the auxiliary activities in the preparation of cows before the milking outside the milking parlour, etc., which cannot be completely included in the optimization model. The variant B1 equipped with a rotary milking parlour with 70 milking stalls has standard technological equipment and 3 milkers are working in it, therefore the time for one milking in the optimum working conditions and help of auxiliary activities is more than 6 hours. The big number of milking stalls is not used efficiently, if the milkers follow exactly the milking procedure and do all working operations without ony help.

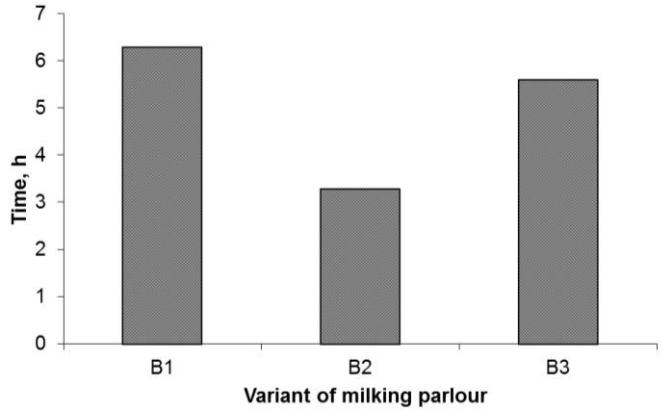

Figure 3. Time of one milking in the rotary milking parlour with 70 milking stalls on the dairy farm 1,850 cows and 3 milkers (B1), and 6 milkers (B2) and at the dairy farm 3,300 cows with 6 milkers (B3).

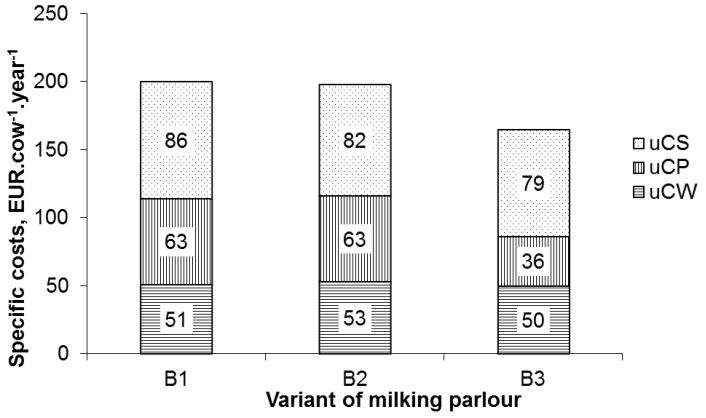

Figure 4. Specific costs of milking in the rotary milking parlour with 70 milking stalls on the dairy farm 1,850 cows and 3 milkers (B1), and 6 milkers (B2) and at the dairy farm 3,300 cows with 6 milkers (B3).

The variant B2 has the same level of technological equipment but with 6 milkers, so this variant is more efficient, and big number of milking stalls enables to 6 milkers to work in this parlour. Time of milking can be reduced (Fig. 3) and final specific costs of milking parlour $^{u}C_{MP}$ have similar level like the varian B1 (Fig. 4). The variant B3 is also the same rotary milking parlour with 70 milking stalls but used for the large dairy farm with 3,300 cows which seems to be efficient solution (Figs 3 and 4). It results in the lowest specific $^{u}C_{P}$ costs. The use of this milking parlour with 6 milkers results in the acceptable time of one milking.

Generally, the organisation of milking process in this huge type of dairy farm is not easy. It is very probable that in the practical application can be time of milking longer than results of this calculation due to the time loses and practical problems with the organisation of movement dairy cows in the farm and auxiliary activities in preparation of cows for milking, etc.

CONCLUSIONS

The time for milking and the final specific direct costs are the main parameters which enable evaluation and choosing of suitable milking parlour for the dairy farm. Both previous mentioned parameters in proposed methodology include the main technical parameters, indicators of labour productivity and economic criteria which can be used for determination of optimal parameters of milking parlour.

Calculation for the first farm with a capacity of 300 cows showed that in the case of rotary milking parlour with 32 milking stalls total specific direct costs per milking per cow and year would be by 25% higher than in the case of Side by Side milking parlour 2 x 12, but the time for milking would be reduced by about 25%.

The second farm with capacity of 1,850 cows is equipped with a rotary milking parlour with 70 milking stalls and with three milkers. Six milkers would bring shortening of one milking from 6.3 h to 3.3 h while preserving approximately the same total specific direct costs per milking per cow and per year. This milking parlour could be used also for the planned increase in capacity at farm to 3,300 cows. Time of one milking would be 5.6 hours, but total specific direct costs per milking per cow and per year would be reduced by 18%. Increased capacity of dairy farm obviously enables to reduce the final specific direct costs for milking.

It is advantage that this model allows, unlike the calculations solved earlier by other authors, to change all basic parameters of the construction and operation of the milking parlour on dairy farms. The preliminary calculations in the preparatory phase before developing a project enable to evaluate (positives and negatives) various solutions of milking parlours. The evaluation of existing milking parlours in the farms can help to improve the milking process and operations from the point of view of either technical improvement or improved activity of milkers.

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Bacterial endophytes of grapevine (*Vitis vinifera* L.) as promising tools in viticulture: isolation, characterization and detection in inoculated plants

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Abstract. Bacterial endophytes may positively influence the host plants, and the search for new strains with beneficial properties is a promising research direction. We isolated culturable endophytic bacterial strains from cuttings of grapevine of four cultivars, identified and characterized their physiological properties and studied colonization process and localization sites of the introduced DsRed-labeled strain. The taxonomic diversity of microorganisms isolated from the inner tissues of grapevine was identified based on the analysis of the 16S rRNA gene fragments. Several promising strains of endophytic bacteria were isolated. DsRed+ phenotypes were obtained by transformation. Their introduction into grapevine plants made it possible to reveal their localization in the plant vascular tissues.

Key words: grape (*Vitis vinifera* L.), bacterial endophytes, 16S rRNA gene, colonization activity, DsRed- labeling, CSLM.

INTRODUCTION

Internal tissues of plants harbour numerous bacteria, which may be involved in neutral, positive or negative interactions with the host plant (Lipka & Panstruga, 2005, Tichonovich & Provorov, 2009). Bacterial endophytes may stimulate plant growth (Taghavi et al., 2009), increase the availability of mineral nutrition elements (Malinowski et al., 2000), biologically fix nitrogen (de Bruijn et al., 1997; Doty, 2011), suppress the development of pathogenic microorganisms (Kloepper et al., 2004; Melnick et al., 2008; Bae et al., 2011) and enhance the immune response of plants (Kloepper et al., 2004; Melnick et al., 2008). Colonizing the econiches of phytopathogens, beneficial bacterial endophytes may act as biocontrol agents (Berg et al., 2005). The search for new endophytic microorganisms with beneficial properties is a promising research direction (Ryan et al., 2008; Chebotar et al., 2015).

In particular, endophytic bacteria may serve as biocontrol agents of various fungal and bacterial diseases of grapevine. The presence of endophytic bacteria from the genera *Streptomyces, Pseudomonas, Bacillus* and some others decreased the negative impact of

the phytopathogens *Fusarium oxysporum*, *Erysiphe necator*, *Plasmopara viticola* and *Xylella fastidiosa* on the grapevine plants (Compant et al., 2013).

In this work, we isolated culturable strains of bacteria from the grapevine cuttings of four cultivars and characterized their physiological properties. The main aim of this work was to identify potentially beneficial endophytic bacterial strains and study the colonization pattern of introduced strains.

MATERIALS AND METHODS

Plant material, bacterial and fungal strains, media

We isolated bacterial endophytes from plants of grapevine (*Vitis vinifera* L.) of four cultivars (Fetyaska belaya, Rkatsiteli, Muskat belyi and Muskat chernyi) sampled in Astrakhan region and Krasnodar region (Russia). Analyzed in this paper 4 grapes cultivars according to their areas of origin and distribution, as well as the aggregate of botanical, morphological and anatomical characteristics and properties related to the Euro-Asian group. According to the classification of A.M. Negrul (Negrul, 1968) this Euro-Asian cultivars by geographical distribution and biological characteristics relate to the one ecological-geographic group – the group of the Black Sea coast – *Vitis vinifera convar. pontica Negr*. The experimental period included 2 years (2014–2015) investigations, sample collection procedure was carried in the end of May in the same geographical points. Cuttings of lignified grapevine stems (30–35 cm in length) were placed into sterile polythene bags, transported to the laboratory and used for isolation of endophytic bacteria.

Fungicidal properties of the isolated strains were tested on the phytopathogenic and toxicogenic fungi *Fusarium oxysporum* (*Fusarium* wilt of grapevine), *Botrytis cinerea* (grey mold) and *Alternaria* sp. (Alternaria rot) (strains from the All Russia Research Institute for Plant Protection (VIZR) collections, kindly provided by Dr T. Yu. Gagkaeva). Antibacterial properties of the isolated strains were tested on five strains of common phytopathogenic bacteria: *Pseudomonas syringae* 213, *Erwinia carotovora var. atroseptica* 822, *Erwinia carotovora var. atroseptica* 3304, *Pseudomonas syringae pv. atrofaciens* p-88, *Xanthomonas campestris* 7604 (strains from the VIZR collections, kindly provided by Dr A.M. Lazarev).

The seeds of cress (*Lepidium sativum* L.) cultivar 'Vesenniy' and tomato (*Solanum lycopersicum* L.) cultivar 'Ataman' were used as the test objects for study of bacterial plant growth promotion (PGP) – properties, generally auxin production and colonization activity.

We used agarized and liquid media: R2A (Hycase SF – 0.5 g l^{-1}; yeast extract – 0.5 g l^{-1}; peptone – 0.5 g l^{-1}; glucose – 0.5 g l^{-1}; starch – 0.5 g l^{-1}; potassium hydrophosphate (K_2HPO_4) – 0.3 g l^{-1}; magnesium sulfate ($MgSO_4 \cdot 7H_2O$) – 0.024 g l^{-1}; sodium pyruvate ($CH_3COCOONa$) – 0.3 g l^{-1}; agar-agar – 17 g l^{-1}), 2% potato agar (potato broth (20 g of potato per 1 l of water) and agar-agar – 17 g l^{-1}), potato-dextrose agar PDA (Difco, USA), Muromtsev medium (glucose – 10 g l^{-1}; asparagine – 1 g l^{-1}; potassium sulfate – 0.2 g l^{-1}; magnesium sulfate ($MgSO_4 \cdot 7H_2O$) – 0.2 g l^{-1}; maize extract – 0.02 g l^{-1}; calcium orthophosphate ($Ca_3(PO_4)_2$) – 4.5 g l^{-1}; agar-agar – 20 g l^{-1}).

Isolation and identification of bacterial endophytes

Endophytic bacteria were isolated from grape plants using an original method of surface sterilization of plant samples. Four to five lignified grapevine cuttings with a length of 10–15 cm were weighed, placed in sterile 500 mL flask, washed three times in sterile water, purified of excessive tissue and placed for a few seconds in 70% ethanol. Then the cuttings were sterilized for 10 min in 10% hydrogen peroxide and washed five times in sterile water. Surface-sterilized cuttings were cut under sterile conditions, pieces of xylem and core were plated onto R2A medium (Difco, USA). The plates were incubated for 5 days at 20 °C, the isolates obtained by plating were purified and stored at -80 °C in sterile broth containing 20% glycerol.

Bacterial DNA was extracted from the isolated bacterial strains using lysis by lysozyme and SDS, protein precipitation by 3M sodium acetate, purification by phenol:chloroform:isoamyl (24:24:1) and DNA precipitation by isopropanol. Briefly, portions of the 16S rRNA genes were obtained via PCR amplification with primers 27 fm (5′-AGA GTT TGA TCM TGG CTC AG-3′) and 1522R (5′-AAG GAG GTG ATC CAG CCG CA-3′) (Weisburg et al., 1991). The amplified DNA fragments were digested with the two nucleases Msp I and Hae III. The resulting fragments were separated on a 2% agarose gel and the profiles of the endophytic strains were compared. For nucleotide sequence determination, PCR products were separated on a 1% agarose gel, recovered and purified from agarose using a QIAquick PCR Purification Kit (QIAGEN GmbH, Hilden, Germany). Sequencing was performed according to the manufacturer's recommendations for GS Junior (Roshe, The Switzerland). Similarity searches in GenBank were performed using BLAST (http://www.ncbi.nlm.nih.gov/blast/; Altschul et al., 1997).

Physiological activity of bacterial endophytes

Endophytic bacteria that could produce auxins were cultured on R2A liquid nutrient medium with the addition of 500 мg l^{-1} of L-tryptophan. To identify strains producing much indole-3-acetic acid (IAA) and its derivatives, we used Salkowski reagent, which gives a characteristic pinkish-red staining with IAA (Salkowski, 1885; Glickmann & Dessaux, 1995). IAA is the main hormone of the auxin type responsible for phytostimulation.

Antagonistic activity of the isolated strains against fungal phytopathogens was tested using the well method (Magnusson & Schnurer, 2001) on PDA medium. Antagonistic activity of the isolated strains against bacterial pathogens was tested with the use of agar blocks method (Zenova et al., 2002) on 2% potato agar.

Protease, amylase and lipase activity were revealed with the use of standard techniques for assessing bacterial enzyme activity (Netrusov et al., 2005; Tepper & Shilnikova, 2005). Pectinase activity was studied in the medium with bromothymol blue as an indicator (Jakob et al., 2009). Cellulase activity was tested in the medium with microcrystalline cellulose following Kasana et al. (2008). Solubility of poorly soluble phosphorus compounds was revealed in the Muromtsev medium (Netrusov et al., 2005) with the addition of 4.5 g of $Ca_3(PO_4)_2$.

To reveal growth-stimulating effect, the strains under study were grown on liquid R2A medium up to the optical density of 1 at a wavelength of 600 nm. After that, the nutrient medium was washed off the culture with the physiological solution (0.85% NaCl solution) and the optical density was made to reach 0.5. Seeds of cress (*Lepidium*

sativum) were soaked in the suspension of bacterial cells at 1:10, 1:100 and 1:1000 dilutions for 15 min. Three days later, root length and shoot length was measured in germinated seeds. Sterile distilled water without bacterial cells was used as the control. Data on the root and the shoot length were statistically processed with the help of the one-factor dispersion analysis using DIANA software (ARRIAM, Russia).

Construction of DsRed-tagged *Pseudomonas* sp. 2ES (strain 2ES-DsRed)

Plasmid pMP4662 (Bloemberg et al., 2000) was transformed into *Pseudomonas* sp. 2ES by electroporation. Electrocompetent cells were prepared according to Choi (2006). For electroporation, 500 ng of plasmid DNA purified by gel electrophoresis was mixed with 100 µl of electrocompetent cells and the mixture was transferred to a 2 mm gap width electroporation cuvette. Immediately after applying a pulse (settings: 25 µF; 200 Ω; 2.0 kV on a Bio-Rad GenePulser; Bio-Rad), 1 ml of room temperature LB medium was added. The cells were transferred into a glass tube and shaken for 2 h at 37 °C. The cells were harvested in a microcentrifuge tube and 100 µl of the harvest was plated on an LB + Tc 20 мg l^{-1} plate. The plates were incubated at 37 °C until colonies appeared (usually within 24 h). Cells pulsed without the addition of DNA served as controls.

After transforming the plasmid to *Pseudomonas* sp. 2ES-DsRed by electroporation, the stability of the plasmids in the rhizosphere (without the antibiotic pressure) was assessed. For this, tomato seedlings were inoculated with the 2ES-dsRed derivatives as described below and their growth was studied in a gnotobiotic quartz sand system. After seven days, bacteria were isolated from the root tip as described elsewhere (Simons et al., 1996) and plated on LB agar plates without antibiotics.

Introduction and visualization of strain 2ES-DsRed within plant tissues

Bacteria harboring plasmids with DsRed genes were examined using confocal scanning laser microscope Carl Zeiss LSM 510 META NLO (Carl Zeiss, Germany). Filter sets tailored to the specific chromophores were used for DsRed, 510-nm excitation with 550–575 nm emission. Tomato roots colonized by *P.* sp. 2ES-DsRed after 5 days of growth in the gnotobiotic sand system were washed in phosphate-buffered saline (PBS) to remove sand particles and mounted in PBS on an attached coverslip. Samples were examined under the confocal scanning laser microscope.

Pseudomonas sp. 2 ES-DsRed strains were cultivated on liquid LB + Tc 20 mg l^{-1} at 28 °C and 200 rpm for 48 hours. The culture of bacteria was added to the fresh inoculum based on PNS solution to achieve the final concentration of 10^5 CFU ml^{-1}. Grape cuttings (Rkatsiteli cultivar) were placed in cylindrical glass bottles filled with PNS solution (1/5 of the vessel volume) containing the inoculum of *Pseudomonas* sp. 2ES-DsRed (Fig. 1, B). The cuttings were kept in the bottles with the inoculum in a climate chamber at a temperature of 20 °C for 7 days. Sections with a thickness of 70–80 µm were made using the vibratome and examined under the confocal scanning laser microscope.

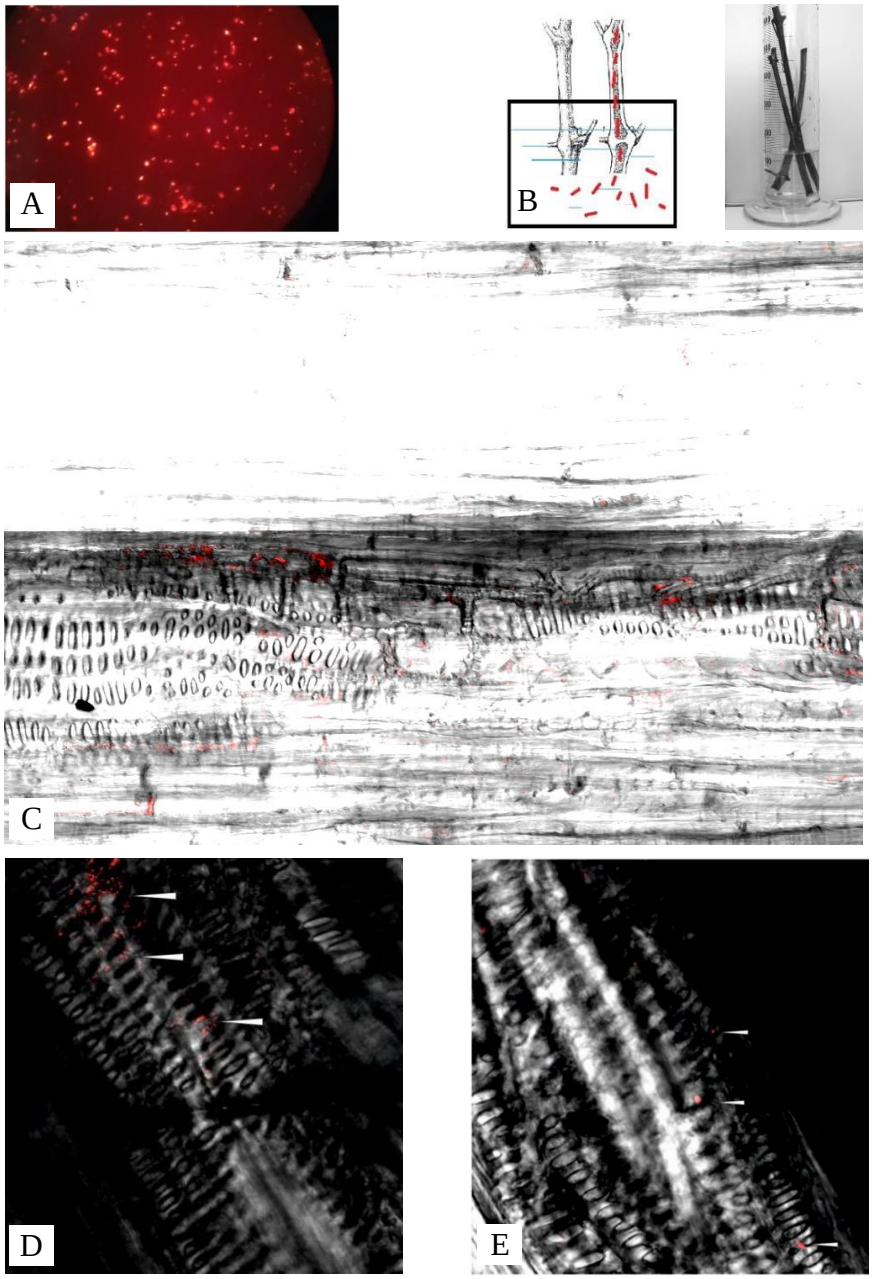

Figure 1. Introduction and localization of DsRed- labeled strain *Pseudomonas sp.* 2ES+DsRed in grapevine stems: A – Fluorescence of the cells of the transformed strain *Pseudomonas sp*. 2ES+DsRed. Epiluminescent microscopy. 1000X. B – Scheme of introduction of the biocontrol strain *Pseudomonas sp*. 2ES+DsRed; C – The main area of localization of the introduced strain *Pseudomonas sp.* 2ES+DsRed in pitted vessels of the grapevine vascular tissue; D, E – microcolonies and single cells of *Pseudomonas sp.* 2ES+DsRed in pitted vessels of grapevine. Bacterial cells were detected as a red signal (arrows). Confocal scanning laser microscopy with detection of DsRed emission in the wavelength of 550–575 nm, magnification 400X, 200X.

RESULTS

We isolated more than 40 culturable bacterial strains from the tissues of grapevine plants belonging to of four cultivars and characterized their morphological and cultural features and cultivation requirements. Most of the isolated bacteria (> 80%) were Gram-positive. They were represented by very small (about 1.5–2.0 μm) rounded or elliptic cells to. On the R2A medium they formed dry flat colonies with a scalloped edge. The colonies were opaque and beige or milky whitein color and could be fast or slowly growing.

The taxonomic diversity of the microorganisms isolated from the inner tissues of grapevine (four cultivars) was identified at the molecularly with level based on the analysis of the 16S rRNA gene fragments. A characteristic feature of bacterial populations of grapevine of different cultivars and different geographical origin was the presence of the genus *Bacillus* (Table 1), which formed characteristic bacillar colonies on the media. Species of this genera may be specific of the endophytic bacterial community of grapevine (Baldan et al., 2016).

Most of the isolated strains showed a complex activity against the test cultures of phytopathogens. The highest antibacterial and antifungal activity was shown by *Bacillus amyloliquefaciens* and *B. atrophaeus*, which suppressed most of the test cultures. *B. subtilis*, *B. pumilus*, *B. megaterium* and *Pantoea vagans* were moderately active against phytopathogens. Several strains with a complex biocontrol activity – namely *B. atrophaeus* SP14, *B. subtilis* Sof4, *B. amyloliquefaciens SP2*, and *B. amyloliquefaciens SP16* – were chosen as promising candidates for the development of phytoprotective biopreparations to be used in viticulture.

Five isolates (*Pantoea vagans* Sof1, *B. megaterium* Sof3, *B. megaterium* Sof5, *B. thuringiensis SP11*, and *Pseudomonas sp*. 2ES) showed plant growth promoting activity. These strains synthesized auxins on the medium with L-tryptophan and stimulated the development of cress seedlings, increasing the length of roots by 27–79% as compared with non-inoculated plants. These bacterial strains had a rather hydrolytic enzyme activity. Strains actively releasing proteinases, amylases, lipases, pectinases and cellulases were identified.

Transformation of several promising strains of endophytic bacteria belonging to the genus *Pseudomonas* (chosen due to the complex of physiological and beneficial properties) allowed to obtain DsRed+ phenotypes. We chose the transformant of strain *Ps. sp*. 2ES+DsRED carrying the plasmid pMP4662, which was as good as the initial native strain in respect of fungicidal, growth-stimulating and colonization properties (Table 2). The cells of this transformant showed a bright fluorescence (maximum excitation at a wavelength of 554 nm, maximum emission at 586 nm). The image of a pure culture of the obtained transformant of *Pseudomonas sp.* 2ES+DsRED under an epiluminescent microscope is shown in Fig. 1A.

The introduction of the marked strain *Pseudomonas sp*. 2ES+DsRED into the vegetative parts of grapevine plants made it possible to reveal its localization in the vascular tissue (Fig. 1, C, D, E). *Pseudomonas sp*. 2ES+DsRED were localized in pitted vessels of grapevine shoots, as single cells or in small groups. The strain was mobile and could circulate in the vessels.

Table 1. Taxonomic position, beneficial properties and physiological characteristics of the most promising strains of endophytic bacteria associated with grapevine

Grape cultivar	Strain	Fungicidal activity	Bactericidal activity	Production of auxins, on L-tryptophane containing medium	Enzymatic activity	Ability to dissolve phosphates ($Ca_3(PO4)_2$)	Plant growth promoting activity
Black Muskat	*Pantoea vagans* Sof1	Al	Pst,	+	Pc	-	+79%
	Bacillus sp. Sof2	-	Pst,Psa,Clm	-	-	-	-
	B. megaterium Sof3	-	Pst, Psa	+	A,L	+	+69%
	B. pumilus SP7	Fo	-	-	P	+	-
	B. atrophaeus SP13	Al,Bc,Fo	Pst	-	A,C,L,P,Pc	+	-
	B. atrophaeus SP14	Al,Bc,Fo	Pst, Ec	-	A,C,L,P,Pc	+	-
	B. subtilis SP15	Al,Bc,Fo	Ec, Xc	-	A,C,L,P,Pc	+	-
Fetyaska	*B. subtilis* Sof4	Al,Bc,Fo	Pst, Clm	-	A,P	-	-
	B. megaterium Sof5	-	Pst	+	-	-	+61%
	B. subtilis SP1	Al,Bc,Fo	Pst,Psa,Ec,Xc	-	A,C,L	+	-
	B. amyloliquefaciens SP2	Al,Bc,Fo	Pst,Psa,Ec,Xc	-	A,C,L	+	-
	B. amyloliquefaciens SP3	Al,Bc,Fo	Pst,Psa,Ec,Xc	-	A,C,L	+	-
	B. amyloliquefaciens SP4	Al,Bc,Fo	Pst,Ec,Xc	-	A,C,L,P	+	-
	B. amyloliquefaciens SP5	Bc,Fo	Pst,Psa,Ec,Xc	-	A,C,L,P	+	-
	B. amyloliquefaciens SP6	Bc,Fo	Pst,Psa,Ec,Xc	-	A,C,L,P	+	-
	B. amyloliquefaciens SP8	Al,Bc,Fo	- Pst,Psa,Ec,Xc	-	A,C,L,P	+	-
	B. pumilus SP9	Fo	-	-	P	+	-
	B. pumilus SP10	Bc,Fo	-	-	P,Pc	+	-
	B. thuringiensis SP11	Bc,Fo	-	+	A,C,L,P	+	+38%
	B. pumilus SP12	Al,Bc,Fo	-	-	A,P	+	-
	B. amyloliquefaciens SP16	Al,Bc,Fo	Pst,Psa,Ec,Xc	-	A,C,L,P	+	-
	B. subtilis SP17		Ec	-	A,C,L,P	+	-
Rkatsiteli	*Pantoea vagans* Sof6	Al,Bc,Fo	Pst, Psa, Ec,	-	A,L,P	-	-
	Pseudomonas sp. 2ES	Al,Bc,Fo	-	+	A,P	-	+27%
	B. amyloliquefaciens SP18	Al,Bc,Fo	Pst,Ec,Xc	-	A,C,L	+	-
	B. amyloliquefaciens SP19	Bc,Fo	Pst,Ec,Xc	-	A,C,L	+	-
	B. amyloliquefaciens SP20	Bc,Fo	Pst,Ec,Xc	-	A,C,L	+	-
	B. amyloliquefaciens SP21	Bc,Fo	Pst,Ec,Xc	-	A,C,L,P	+	-
Muskat	*B. amyloliquefaciens* 22	Bc,Fo	Pst,Psa,Ec,Xc	-	A,C,L,P	-	-

Note: Fungicidal activity against: Al – *Alternaria sp*., Bc – *Botrytis cinerea*, Fo – *F. oxysporum*; bactericidal activity against: Pst – *Pseudomonas syringae pv. tomato*, Psa – *P. syringae pv. atrofaciens*, Ec – *Erwinia carotovora pv. atroseptica*, Xc – *Xanthomonas campestris*, Clm – *Clavibacter michiganensis pv. sepedonicum*; enzymatic activity: A – amylase, C – cellulose, L – lipase, P – protease, Pc – pectinase; Plant growth promoting activity – % increment of root length of radish seedlings.

Table 2. Comparative characteristic of physiological-biochemical properties of the genetically modified strain *Pseudomonas. sp*. 2ES+DsRed and the native form

Characteristic	*Pseudomonas sp*. 2ES+DsRed	Native strain *Pseudomonas sp*. 2ES
Number of bacteria in 24 h culture on LB medium, Cfu ml^{-1}	$750 \cdot 10^6$	$775 \cdot 10^6$
Zone of inhibiting growth of *F. culmorum* (5 day), mm	23	24
Zone of inhibiting growth of *F. oxysporum* (5 day), mm	26	25
Production of auxins, on L-tryptophane containing medium	+	+
Number of bacteria in rhizosphere of tomato plants, Cfu g^{-1} rhizosphere substrate	$250 \pm 25 \cdot 10^4$	$137 \pm 43 \cdot 10^4$
Number of bacteria on the roots of tomato plants, Cfu cm^{-1} root length	$37 \pm 5 \cdot 10^4$	$45 \pm 8 \cdot 10^4$

DISCUSSION

In this work about 40 strains of culturable bacteria were isolated from the endosphere of grapevine. Gram-positive spore-forming bacteria from the genus *Bacillus* dominated among the isolated strains. Their abundance made up 89.6%.

Numerous previous studies have shown that both Gram-positive and Gram-negative bacteria can be present in the endosphere of grapevine. For instance, strains of *Bacillus spp., Pseudomonas spp. and Burkholderia spp*. were isolated from leaves and shoots of grapevine (West et al., 2010). Numerous and diverse species of *Bacillus, Pseudomonas* and *Pantoea* were isolated from grapevine flowers, berries and seeds (Compant et al., 2011), with the colonization niches visualized by FISH method.

Taxonomic diversity of bacterial endophytes of grapevine plants was previously studied using culture independent technique (detection by LH-PCR) (Bulgari et al., 2014) and bacteria previously reported as biocontrol agents (*Burkholderia, Methylobacterium, Sphingomonas* and *Pantoea*) were identified. Similar taxa have also been found with the use of ARISA fingerprinting and pyrosequencing of 16S rDNA (Campisano et al., 2014) and high levels of the dominant genera (*Ralstonia, Burkholderia* and *Pseudomonas*) were detected in all the samples. Bacterial endophytes from genera *Bacillus, Pseudomonas* and *Pantoea* have also been found in the internal tissues of grapevine plants (Andreolli et al., 2016). Antifungal activity analysis showed that two of the *Bacillus* strains possess growth antagonistic effect against all the tested fungal strains.

In this work, we screened bacterial endophytes of grapevine for economically valuable properties such as antagonism against fungal and bacterial phytopathogens, ability to produce IAA, mineralization of poorly soluble phosphorus compounds, enzymatic activity and the ability to stimulate plant growth. As a result, we chose a number of strains with a complex of beneficial properties: *B. amyloliquifaciens, B. atropheus, B. subtilis* and *B. megaterium* which are also described as grape endophytes by some authors (Huang et al., 2011; Liu et al., 2014; Wang & Liang, 2014; Wu et al., 2015).

The strain *B. thuringiensis* SP11 is of special interest. Its introduction into grapevine plants might make it possible to control insect pests (Monnerat et al., 2009; Tanuja et al., 2013; Tao et al., 2014) by purely biological means without the genetic modification of plants with the use of Bt genes.

Gram-negative strains, in particular, those from the genus *Pseudomonas*, are also promising in respect of PGP-properties. These common microorganisms might be good biocontrol and growth-stimulating endophytic agents of grapevine (Kilani-Feki et al., 2010; Verhagen, 2010).

In our study, we showed that an introduced DsRed-markered strain with biocontrol and growth-stimulating properties may successfully colonize pitted vessels (xylem) of grapevine shoots. While the localization of native strains in the leaves (Lo Piccolo et al., 2010) and berries (Compant et al., 2011) has been shown before, the introduced strain was visualized in the xylem of grapevine for the first time.

Endophytic PGP bacterial strains maybe used to colonize the vascular tissues of grapevine cuttings before rooting. These strains may biocontrol phytopathogens and stimulate plant growth during rooting and further development. In this way, self-sufficient plant-microbial systems protected from bacterial and fungal infections would arise. The future studies will include the investigation of colonization strategy by native (not modified) bacillar strains in the inner tissues of grapes cuttings with FISH methods. Also we plan to study the taxonomy of bacterial endophytes from Euro-Asian grape cultivars using metagenome analysis of bacterial community.

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Application of conventional HPLC RI technique for sugar analysis in hydrolysed hay

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Abstract. To determine the potential biofuel yield and necessary technological parameters a known concentration and type of fermentable sugars should be produced during chemical or biological extraction from lignocellulose. The most popular method for sugar interpretation and quantification is liquid chromatography (HPLC) using refractive index (RI) detector. The aim of this research was to show the applicability of the high–performance liquid chromatography using refractive index (HPLC RI) technique for sugar interpretation in hydrolysed hay and possible solutions for optimisation of this method. Analysis of hydrolysed hay with standard additive showed low recovery of sugar concentrations and inconsistencies with dinitrosalicylic acid (DNS) method, which was mostly due to low separation of peaks of these sugars on the chromatograms. As result HPLC RI method was useful for qualitative analysis of sugars only, not for its quantification.

Key words: lignocellulosic biomass, sugar determination, DNS method, HPLC RI.

INTRODUCTION

Agricultural biomass is one of the most sustainable sources for biofuel production (Chandra et al., 2012). The first stage in biofuel production is chemical or biological extraction of sugars from recalcitrant lignocellulose (Hahn-Hägerdal et al., 2006). Different types of sugars can be extracted in solution through the process of hydrolysis depending on source material: monosaccharides, oligosaccharides, polysaccharides, neutral sugars, acidic sugars, amino sugars, sugar alcohols, and their various isomers (Solomons, 1980). To determine the potential biofuel yield, most appropriate product type and technological parameters, all produced sugars should be accurately quantified and interpreted.

Various methods can be used for sugar determination and quantification depending on the sugar source and technological process: (i) the total sugar content in cotton fibres by spectrophotometric method (ISO, 2014); (ii) the reference method for the determination of lactose content in milk (ISO, 2007); (iii) dinitrosalicylic acid (DNS) method to determine total isolated reducing sugar concentration in solution. These methods are relatively simple, fast and with good precision, however, most of them do not offer any discrimination among sugar types (pentose and hexose sugars). Quantification of specific sugar concentrations in any solution can be performed

withhigh–performance liquid chromatography (HPLC) (Solomons, 1980; Shaw, 1988; Kelebek et al., 2009; Nefolar et al., 2010; De Goeij, 2013).

To perform sugar HPLC analyses different detection systems can be used: (i) UV–visible detection; (ii) fluorescence detection; (iii) electrochemical detection or (iv) evaporative light scattering detectors (Solomons, 1980; Baranenko et al., 2014). Some of thesedetectors are better for determination and description of sugars found in regions near 190 to 195 nm, otherfor components that emit fluorescence. However, it is known that generally sugars are not fluorescent, thus, the selectivity of the previous mentioned detection systems is not very high and the equipment is difficult tooperate.The most popular method for sugar interpretation is liquid chromatography (HPLC) using refractive index (RI) detector due to the simplicity of analysis, lower costs of equipment and reagents when compared with other techniques (Ellefson, 2003; De column and detector must be found (De Goeij, 2013).

The aim of this research was to study the applicability of the high–performance liquid chromatography using refractive index (HPLC RI) technique for sugar interpretation in hydrolysed hay and possible solutions for the optimisation of this method.

MATERIALS AND METHODS

Sugar production from lignocellulosic biomass

Hay or straw biomass was grounded by grinder (Retsch GM200) to obtain fractions < 0.5 cm. Further the biomass was diluted in 0.05 M sodium citrate buffer (3% w/v, pH 5.5) and boiled for 5 min to eliminate any indigenous microorganisms.

For enzymatic hydrolysis, laboratory prepared enzyme (0.2 FPU ml^{-1}, Mezule et al., 2015) or 1% v/v cellulolytic enzyme mixture (Viscozyme L, Sigma Aldrich) was added to the diluted substrates and incubated on an orbital at 30 °C. Samples for sugar analyses were collected after dilution with buffer, after boiling, after 6, 24 and 48 h of incubation. At least 2 samples from each test were collected for sugar measurements.

Sugar standards for HPLC RI analysis

Standard stock solutions of individual sugars (L–(+)–Arabinose, D–(+)–Xylose, D–(–)–Fructose, D–(+)–Mannose, D–(+)–Galactose and D–(+)–Glucose (Sigma Aldrich, Germany)) in methanol (MeOH, Sigma Aldrich) and water (1:1) were prepared in separate volumetric flasks. Working solutions were prepared by diluting the stock solutions with the same solvent to contain (0.5–50 mg ml^{-1} final concentration). Linearity was established by triplicate injections of different concentrations of the standards obtained by dilution in water of the mixture containing the standards. Calibration curves were obtained by plotting peak area versus amount injected.

Determination of total sugar concentration by DNS method

Sugar concentration with dinitrosalicylic acid (DNS) method was performed according to Ghose (1987). In brief, all samples were centrifuged (6,600 g, 5 min). Then 0.1 ml of the supernatant was mixed with 0.1 ml of 0.05 M sodium citrate buffer (mono–sodium citrate pure, AppliChem) and 0.6 ml of DNS (3,5–dinitrosalicylic acid, Sigma Aldrich). For blank control, distilled water was used instead of the sample. Then all samples were boiled for 5 min and transferred to cold water. Further 4 ml of distilled

water was added. Absorption was measured with spectrophotometer at 540 nm (Camspec M501, UK). To obtain absolute concentrations, a standard curve against glucose was constructed.

Sugar determination with HPLC RI

Determination and quantification of specific reducing sugars: arabinose, xylose, fructose, mannose, galactose and glucose was performed by HPLC RI (Perkin Elmer, USA) using COL–AMINO 150 x 4.6 mm column (Perkin Elmer, USA). The analysis was performed at 35°C with a flow rate of 1 ml min^{-1} using isocratic elution with 75% acetonitrile (AcN):25% water (H_2O) mixture as a mobile phase. All samples were centrifuged at 4,000 rpm for 10 min (Nuve, Turkey) and the supernatant was filtered through a 0.22 μm PES membrane (Sartorius Stedim Biotech, Germany). Then the filtrate was diluted 10 times before direct injection into the HPLC.

To determine the effect of hydrolysate chemical composition and pre–treatment process on the results, blank samples were used as background. To optimise the sugar separation process the following procedures were checked: changes of oven temperature (from 25 °C to 50 °C), changes of eluent and sample flow rate (from 0.5 to 1 ml min^{-1}) and eluent AcN/H_2O composition (50:50; 75:25; 90:10).

Each sample was tested by triplicate injections. The blank and control solutions were analysed with each series of sample in order to verify the accuracy of the obtained results. Accuracy of each test was calculated by considering the recovery obtained for each compound at 10 mg l^{-1} concentration level in control solution (Table 1) and the mean value was calculated (coefficient of variation CV < 10%). HPLC results (retention time and resolution) of each specific reducing sugar in samples were compared with the retention time and resolution of sugar standards.

Statistical data analysis

General MS Excel statistical data analysis was used for data processing. To determine if the data sets are significantly different or not, t–test analyses (MS Excel 2007) were performed for two tailed distributions. Probabilities of ≤ 0.05 were considered as significant. HPLC Flextar programme Chromera Manager 3.4.0.5712 was used for chromatogramm data processing.

RESULTS AND DISCUSSION

In this study, the dinitrosalicylic acid (DNS) method was used to determine total reducing sugar concentration and the HPLC RI method for identification and quantification of specific reducing sugars isolated from hydrolysed hay. The HPLC system used in this study was equipped with gradient pump, column oven, RI detector and conventional amino column (COL–AMINO 150 x 4.6 mm).

The results obtained for the method validation are shown in Table 1. Linearity and range of application of the method were measured by determining the calibration curve by the linear models of L–(+)–Arabinose, D–(+)–Xylose, D–(–)Fructose, D–(+)–Mannose, D–(+)–Galactose and D–(+)–Glucose respectively.

Table 1. Linear regression parameters obtained from the sugar standard calibration curves and control tests

Sugar	Regression Equation	R^2	MDL, mg l^{-1}	% RSD (RT)
D–(+)–Glucose	y = 42.7944x – 9.2866	0.9999	0.28	0.36
D–(+)–Xylose	y = 29.9900x – 4.7208	0.9998	0.19	0.23
D–(–)–Fructose	y = 46.2561x + 0.2385	0.9999	0.04	0.24
D–(+)–Mannose	y = 22.9531x – 4.1942	0.9999	0.31	0.11
L–(+)–Arabinose	y = 22.6182x – 1.7041	0.9995	0.21	0.26
D–(+)–Galactose	y = 26.7419x + 9.3517	0.9997	0.35	0.33

The minimal detection limit (MDL) was obtained by the dilution of standards to evaluate the minimal concentration of sugars that can be determined with–in this study. Retention time relative standard deviation (RSD RT) was calculated by HPLC Flextar programme Chromera Manager 3.4.0.5712, which was used for chromatogram data processing by using the retention time of individual sugars during the calibration injections. The obtained detection limits, regression coefficients (R^2) and RSD RT indicated that the system is technically suitable for hay analyses.

Analysis of individual sugar standard solutions in water showed that retention time (RT) of arabinose was 4.7 min, xylose–4.0 min, fructose–4.7 min, mannose–5.3 min, galactose–5.5 min and glucose–5.4 min. It was possible to perform calibration by using single sugar standard solution or with a mixture of multiple sugars with different RT: mix 1–xylose, glucose and fructose (Fig. 1), mix 2–mannose, arabinose and galactose.

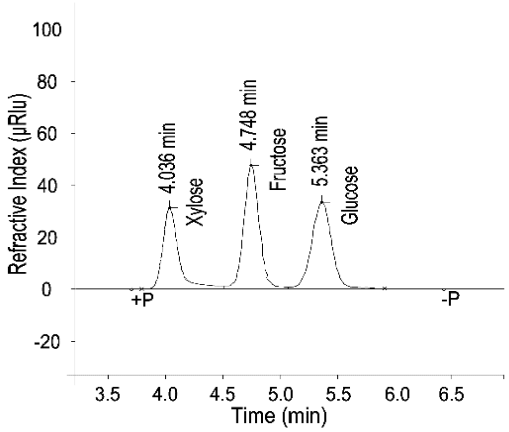

Figure 1. Chromatogram of sugars during calibration of HPLC.

The analysis of hydrolysed hay showed that all sugars (arabinose, xylose, fructose, mannose, galactose and glucose) were found in the enzymatically–hydrolysed hay samples (Fig. 2), but not identified by HPLC programme. The results showed that there is a low separation of peaks of these sugars on the chromatograms. Moreover, the corresponding samples differed from DNS method (28–96%), which always gave lower results. At the same time DNS method presented lower STD values and no difference ($p < 0.05$) among sample repetitions and, similarly as reported before, produced higher

reducing sugar yields from hay biomass than from straw (Mezule et al., 2015). Further evaluation of the HPLC results showed that there is a relatively high concentration of fructose (0–24.4%; MEDIAN 16.9%), mannose (0–48.2%; MEDIAN 31.5%) and galactose (0–33.2%; MEDIAN 0%), which are usually regarded as minor lignocellulosic sugar components (Yabushita, 2016).

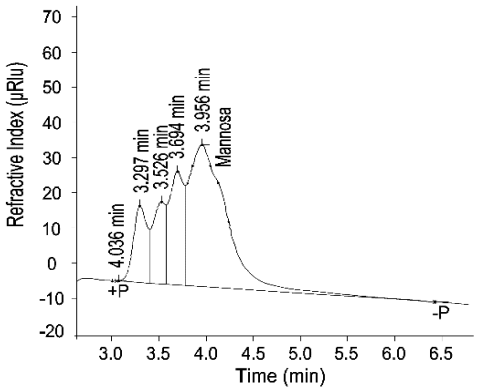

Figure 2. Chromatogram of sugars in hydrolysed hay solution.

To further determine and evaluate the differences in results obtained with both methods, a set of samples were spiked with either glucose or fructose standards. To overcome any changes in sugar standard quality, the spiking was performed after 24 h of hydrolysis and the added volume had a minor effect on overall sample volume (< 1%). The results with DNS method showed that the difference between the spiked samples and unspiked samples and standard concentration does not exceed 5% for both glucose and fructose. At the same time the HPLC data showed that both sugar concentrations in the spiked samples are more than two times higher. Moreover, again high concentrations of fructose (13% v/v) and mannose (34% v/v) were observed in unspiked hydrolysed samples. At the same time glucose yielded only around 22% from all sugars. Low correlation with dinitrosalicylic acid (DNS) method can be explained by low separation of the peaks of these sugars on the chromatograms (Fig. 2).

Different techniques can be used for improving various chromatographic parameters: peak shapes, detection, sensitivity and retention time. Some studies showed that optimization of mobile phase and oven temperature is crucial (Sluiter et al., 2006; Nelofar et al., 2010; Zielinski et al., 2013; Correia et al., 2014). To optimise the sugar separation process the following procedures were performed in this study: changes of oven temperature (25, 30, 35, 40 and 50 °C), changes of RI detector temperature (25 and 35 °C), changes of eluent and sample flow rate (0.5 and 1 ml min^{-1}) and eluent AcN/H_2O composition (50:50; 75:25 and 90:10). Representative results are shown in Table 2.

Table 2. Comparison of RT depending on the oven temperature, RI detector temperature and flow rate

Sugar	RT, min					
	Flow 1 ml min^{-1}; Oven 25 °C; RI 25 °C Injection 5µl	Flow 1 ml min^{-1}; Oven 25 °C; RI 35 °C Injection 5µl	Flow 1 ml min^{-1}; Oven 25 °C; RI 35 °C Injection 20µl	Flow 1 ml min^{-1}; Oven 35 °C; RI 35 °C Injection 5µl	Flow 1 ml min^{-1}; Oven 35 °C; RI 35 °C Injection 20µl	Flow 0.5 ml min^{-1}; Oven 35 °C; RI 35 °C Injection 5µl
arabinose	4.717	3.963	3.970	3.637	3.610	5.468
xylose	4.543	3.643	3.701	3.393	3.351	5.445
fructose	5.131	4.202	4.257	3.840	3.797	5.881
mannose	5.047	4.392	4.404	4.046	4.004	6.111
galactose	6.282	4.287	4.299	4.168	4.107	6.282
glucose	5.732	4.556	4.659	4.183	4.156	6.243

Changes of RT of the sugars in chromatographic column were possible by changing various parameters (oven temperature, flow rate). The results showed that RT increased by 2 min with the decrease of sample flow rates. Injection volume had no effect on the RT, but it can improve detection of low sugar concentrations in samples due to the increase in peak area.

Differences of RT were 0.6–1.2 min in chromatograms at 25 °C and 35 °C temperature of RI detector. RT decreased by 0.1–0.3 min with the increase of oven temperature by 5 °C. Thus, changes intemperature had low effect on the separation of sugars in hydrolysed hay. Changes in eluent AcN/H_2O compositions (50:50; 75:25 and 90:10) had no effect on the chromatogram quality. RT of arabinose and xylose, as well as, fructose and mannose was still too similar.

Some studies (Sluiteretet al., 2008; Correia et al., 2014) showed that detector temperature must be close to column or oven temperature. Other showed better result when difference of temperature between detector and column was very high–column temperature 80 °C and detector temperature 50 °C (Zielinski et al., 2013). Other authors showed that additional chemical reagents are better for optimisation of separation process of sugars in column. Depending on the chromatographic column characteristic sulphuric acid (5–12 mM), ammonium hydroxide (0.04%) or addition of MeOH to solvent solution or changes of eluent AcN/H_2O composition can be used (Hernandez et al., 1998; Sluiter et al., 2008; De Goeji, 2013; Correia et al., 2014). The results of this study showed that for the identification of sugars by HPLC RI in hydrolysed hay it is necessary to examine other HPLC columns and chemical reagents to improve the separation.

CONCLUSIONS

Standard HPLC RI protocol and conventional amino column (COL–AMINO 150 x 4.6 mm) were used for experiments. No specific enhancements were tested in this study. The following general conclusions can be drawn from this research: (i) all sugars (arabinose, xylose, fructose, mannose, galactose and glucose) were found in hydrolysed hay samples by HPLC RI method with amino column (COL–AMINO 150 x 4.6 mm); (ii) analysis of hydrolysed hay showed that there is a low separation of peaks of these sugars on the chromatograms and there is no correlation of sugar concentrations determined with dinitrosalicylic acid (DNS) method; (iii) changes of oven temperature,

flow rate, temperature of RI detector and changes in eluent AcN/H_2O compositions had no effect on the chromatogram quality.

To quantitatively identify sugars withHPLC RI in hydrolysed hay it is necessary to examine other available HPLC columns and chemical reagents to improve the separation.

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Feasibility study of a local power supply system for sparsely populated areas in Estonia

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Abstract. The paper analyzes the reasonability of using an off-grid hybrid power supply system or in other words a local grid for sparsely populated areas as well as the necessary components selection and price development of such system. Typical consumers are selected and all estimations and calculations are based on them. Consumer profiles are set and analyzed as well as different elements of the local power supply grid and the possibility of connecting to the traditional grid. Estonian example is used in this paper as the country lies relatively north and has some remote areas, where local power supply grids can be implemented. All prices in the paper are derived from the Estonian example. Necessity of further study is proposed.

Key words: Distributed generation, PV panels, renewable energy, wind energy.

INTRODUCTION

The world consumes approximately 85 million barrels of oil every day but there are only 1,300 billion barrels of proven reserves of oil (Mi et al., 2011). At the current rate of consumption, the world will run out of oil approximately in the next 40 years (Owen et al., 2010). The emissions from burning fossil fuels increase the carbon dioxide (CO_2) in the Earth's atmosphere (Owen et al., 2010). For example, the total electric energy consumption in Estonia for the year 2015 was 8.1 TWh (Annual Report of Elering, 2015), which represent 3.24 Mt of CO_2 emissions. The increase of CO_2 is a cause of greenhouse effect and climate change. As a consequence, it will lead to instability of ecosystems and, perhaps, rising sea levels. Reducing fossil fuel usage and as a result, reducing carbon emissions are the main goals of humanity nowadays. The production of electricity, a complex process, is not always friendly to the environment, being often connected with burning of fossil fuels (Rassõlkin, 2014). The world today is moving towards smart grids and distributed electricity generation. This is a challenge for traditionally centralized systems, because instead of the big generation units, many smaller units distributed all around the system are starting to emerge (Vaimann et al., 2012). On the other hand, wider usage of renewable energy sources and efficient use of electricity can be the way to solve most of the problems in electricity generation today.

An estimated (WEO, 2015) it is about 1.2 billion people (17% of the global population) did not have access to electricity in 2015. Many more suffer from supply that is of poor quality. More than 95% of those living without electricity are in countries in sub-Saharan Africa and developing Asia, and they are predominantly in rural areas (around 80% of the world total). While still far from complete, progress in providing electrification in urban areas has outpaced that in rural areas two to one since 2000. Using of renewable energy sources gives a broader scale of possible solutions to consumers situated in sparsely populated areas, where no near distribution lines have been built (Vaimann et al., 2013).

The price of electricity consumed from the grid consists of three main parts – costs of electricity production, costs of distribution and taxes, the fourth component that can be also added in electricity bills is regulated policy costs. With the usage of renewable energy sources and dispersed generation a principle possibility emerges, to generate electricity at the spot of consumption. This opportunity can be realized in the situation, when electricity is needed in a place where there are no existing transmission or distribution lines. In certain conditions, usage of electricity generated from local renewable sources can become cheaper, than building a new distribution line to consume electricity from the grid.

To judge if local grid would be the cheaper solution, expenses of both possibilities must be known. This comparison is a simple task when both solutions have already been designed, but as designing those solutions in every individual case is expensive and time consuming, simplified criteria of evaluation must be developed to decide quickly and easily on the feasibility of the mentioned possibilities.

To set the needed criteria, typical electricity consumers and their consumer profiles must be found. After that, it is necessary to calculate expenses of every typical consumer on electricity in case of building a new distribution line. This is followed by designing local electricity supply for each consumer group and calculation of expenses of typical consumers on electricity in case of local grid. In this paper we are going to compare two alternatives of rural power supply: grid extension and local power supply. Finally, the expenses of local grid and new distribution line situations must be compared, generalized and the final criteria must be set. Comparison of the expenses can very well be made according to the price of kWh, but sum of the yearly costs of the consumer could very well be the subject of the comparison as well.

The analysis given in this paper is derived and updated from the authors' previous investigations (Kallaste et al., 2013; Vaimann et al., 2013). As during the past few years there has been major changes in the local electricity market in Estonia, an updated analysis and calculation methodology on the topic is presented.

TYPICAL CONSUMERS AND CONSUMER PROFILES

For the setting of typical consumers, it is obvious that such consumers can be found in remote rural areas, as towns and larger settlements are connected to the traditional grid. In case of rural areas, some remote regions can be found, where people have been living for decades, but due to various reasons, no distribution lines have been built. In Estonia, the estimation of such households reaches to a few hundred. Other similar case are old deserted villages, where people have lived, but moved to towns years ago. Roads

to such places have been preserved to some extent and renovation of them is not too expensive.

On the other hand, there are huge amounts of people in towns, who desire to have a rural cottage to use as a summer house or year-round living during their retirement. Interest towards expanding agricultural production and reusing of former farmlands is rising. All of the listed reasons bring with them a need for electricity in places, where no connection points exist at the moment.

Taken into account the aforementioned possibilities, two possible typical consumers should be chosen under investigation:

1. Summer house, which is used seasonally and thus has a widely varying electricity consumption;
2. Cottage for a single household, that is used the whole year-round.

To simplify the investigation, this paper will be based on only those two consumer types. To carry out the analysis, typical electricity consumption of both the two cases is needed. The important information is the consumed kWh of one day in the time span of one year and the needed maximum power of the two consumers. This is called a consumer profile. When setting a consumer profile, it must not be forgotten, that the profile depends amongst other variables also in the fact if the consumed electricity is coming from the traditional grid and is relatively cheap, or is local grid used, which makes electricity more expensive. In case of local grid devices with lower power consumption such as LED- lights, economical refrigerators etc. can be expected.

Fig. 1 presents a typical annual electricity consumption of a summer house in Estonia. Values are given month by month. Monthly consumption varies 35–80 kWh. Main consumers in the summer house are lighting, refrigerator, electrical oven, partial heating (in addition to traditional wood fueled oven), and water pump. Annual consumption of the summer house is 622 kWh and monthly average is 51.7 kWh.

Fig. 2 presents a typical annual electricity consumption of a cottage used by one family. The household consists of four people living in the house throughout the whole year. Main consumers in this house are refrigerator, TV-set, personal computer, electric oven, lighting and a washing machine. Monthly consumption varies 150–340 kWh. Annual consumption of the household is 2,960 kWh and monthly average is 247 kWh.

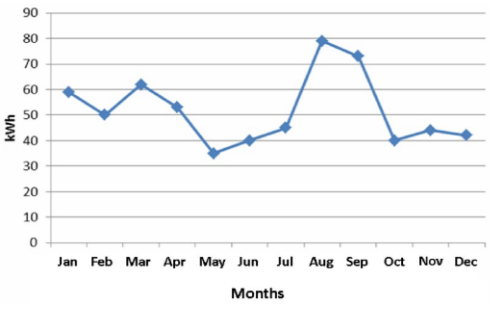

Figure 1. Annual electricity consumption of a summer house presented month by month.

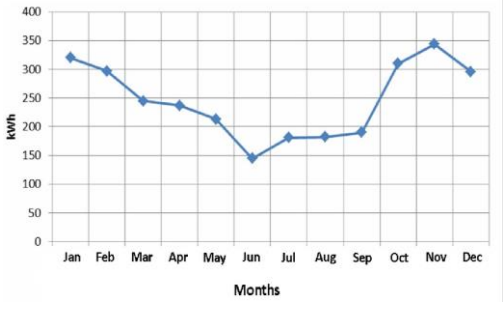

Figure 2. Annual electricity consumption of a cottage used by one family presented month by month.

To estimate the consumption, calculations must be based on either metering data from summer houses or a selection of typical consumers in the aforementioned buildings and their usage times.

Using the data available for authors, a typical summerhouse has the maximum consumption of 80 kWh per month. It can be assumed that in case of local grid connection, the consumption will drop about two times and will be around 40 kWh per month. This means that the daily consumption will be 1.33 kWh in average.

According to Table 1 (Lõokene, 2011), daily consumption of a small one-family cottage based on the typically used devices is 1.75 kWh per day. As the consumption of a summer house is generally smaller, the calculated 1.33 kWh per day seems quite realistic and it means that the weekly consumption is in average 9.3 kWh. The consumption period in a summer house lasts for 7 months from April (week 9) to October (week 44), in total of 35 weeks.

Table 1. Daily consumption of a small one-family cottage based on typically used devices

Device	Power (W)	Daily usage (h)	Daly consumption (Wh)
TV-set	50	3	150
Refrigerator			356
Vacuum cleaner	1,500	0.1	150
Water heater 1.5 l	1,700	0.1	170
Pump	700	0.5	350
Lighting LED 8x11W	88	3	264
Laptop	60	3	180
Washing machine	2,200	0.06	132
TOTAL			**1,752**

Chosen cottage had the annual consumption of 2,960 kWh from the grid and maximum monthly consumption of 340 kWh. Average daily consumption in the maximum consumption month is then 11.33 kWh. There are significant variations between the consumption of individual days, but on the other hand the consumption can be expected to decrease when local supply system is applied. Considering these tendencies and the battery autonomous time of 2 days (less than in the example with the summer house) maximum daily consumption of 10 kWh can be set.

CONNECTION TO NEW DISTRIBUTION LINE

Calculations for consumer connections to new distribution lines are based on the methodology and prices used by the Estonian largest distribution grid company Elektrilevi. According to the company policy, all connection fees for consumers situated further than 400 m from the existing MV line are set separately for each individual case taking local conditions into account.

For the investigation purposes a simplified method for assessing grid connection costs must be found. 0.4 kV line is not suitable for supply in the distance of more than 400 m from the substation (or MV line respectively). MV line must substitute for the 0.4 kV LV cable. All new connections to the grid should be done using earth cables. This is a default requirement in most Nordic countries as well as in Estonia. Lowest voltage MV line suitable for such purposes is 10 kV as previously used 6 kV lines are not being

installed anymore. There is a possibility of using a 1 kV line, but precedent of its usage does not exist in Estonian practice. In this paper calculations for connections are done based on 10 kV lines.

As seen from the consumer profiles described before, load currents are low, so minimal cross-section (25 mm^2) 10 kV earth cable is suitable for servicing the connection. 1 km of such cable line costs 50,000 € according to the information from Elektrilevi. Assuming that the new connection point is situated in the distance of 2 km from the existing MV line and 0.4 kV LV is needed for the consumers, at least two possible alternatives can be thought of.

Firstly, it is possible to use a 2 km MV line and install a substation in the vicinity of the connection point. Other solution would be moving the substation to 1600 m from the existing MV line and use LV cable between the substation and the connection point. As LV cable is cheaper for such load currents, the other option seems more reasonable. Schematic of the substation placement is presented on Fig. 3.

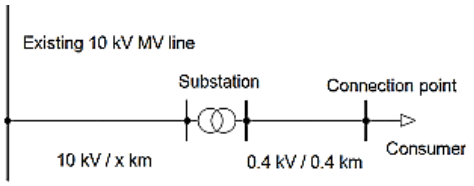

Figure 3. Schematic of substation placement for new connection point.

In the given case, connection to grid further than 0.4 km away is analyzed. This means that the connection fee consists of two parts. First part consists of the price of 10 kV 25 mm^2 MV line, the length of which depends on the exact placement of the connection point and the price of which can be set according to the price per meter and length of the needed line. This can be considered as the variable part of the connection fee.

Second part of the fee is made up from the costs of substation, transformer, switch board, metering system and the 0.4 kV LV line. This part depends on the main fuse amperage. Most used fuse rated values in Estonia are 16 A, 25 A and 32 A, just some of the households using more than 40 A fuses. In Table 2 the prices range for the mostly used valued is presented.

Table 2. Most used fuse rated values in Estonia

Main fuse amperage	Price without VAT	Price includes VAT
16 A	2,080 €	2,496 €
25 A	3,250 €	3,900 €
32 A	4,160 €	4,992 €

Around 15% of additional costs can be expected for other related work such as design and drawings. After this, simplified equation to calculate the costs of connection to the grid can be written:

$$c = [c_c + (d - 400)c_v + c_a\,]K_{VAT} \qquad (1)$$

where: c – connection costs (€); c_c – constant part of the connection fee (€); d – distance of the connection point from the existing MV line (m); c_v – variable part of the connection fee (€ m^{-1} of the 10 kV line); c_a – additional costs of the connection (€); K_{VAT} – value added tax factor (in case of Estonian 20% VAT; K_{VAT} = 1.2)

New kWh price must be found that already considers the costs of new connection to grid. It can be found so that connection costs in a certain time period (e.g. 10 or 30 years) must be divided with consumed electric energy (kWh) and electricity price from sold by provider companies and fixed with contracts must be added to the equation. Following equation can be derived:

$$P_{nem} = P_{con} + \left(\frac{c_c + (d - 400)c_v + c_a}{K_{kWhy}} \right) \frac{K_{VAT}}{c_{el}} \quad (2)$$

where: P_{new} – new price of electricity taking connection fees into account (€ kWh^{-1}); K_{kWhy} – annual consumption in the connection point (kWh y^{-1}); c_{el} – calculated elimination period of the connection fee (years); P_{con} – price of electricity set by the contracts with provider companies (€ kWh^{-1}).

The price paid by consumers for electricity can be divided into three parts: electricity, the network service fee, and state taxes and fees, which cover the renewable energy fee (6.8%), electricity excise (3.9%) and VAT (16.7%). Base transmission fee in package 'Võrk 1' by Elektrilevi 0.054 € kWh^{-1}, electricity price by Eesti Energia AS with fixed value and a single year contract is 0.063 € kWh^{-1} (01.01.2016), including state taxes (Elektrilevi, 2015) and fee the final price will be 0.163 € kWh^{-1}.

Using Eq. 2, different situations can be investigated how the distance of connection point d and different amount of consumed electric energy will affect the electricity price P_{new}. Some of the possibilities are described in Table 3. The main contribution of the new price calculation is that the significant influence on the long term price value is made by the annual electricity consumption. Consequently, the separately connecting of the summer houses to the grid is not reasonable, particularly if they are located far away from the MV line.

Table 3. New prices of kWh (P_{new}) relating from distance of the connection point (d), annual electricity consumption (K_{kWhy}) and calculated elimination period of the connection fee (c_{el})

	P_{new}, € kWh^{-1} (c_{el} = 10 years)							P_{new}, € kWh^{-1} (c_{el} = 20 years)					
	d (m)	$K_{kWh\,y}^{-1}$ (kWh)						d (m)	$K_{kWh\,y}^{-1}$ (kWh)				
		500	1,500	3,000	4,500	6,000			500	1,500	3,000	4,500	6,000
16A	400	0.74	0.35	0.26	**0.23**	**0.21**	16A	400	0.45	0.26	**0.21**	**0.19**	**0.19**
	1,000	7.94	2.75	1.46	1.03	0.81		1,000	4.05	1.46	0.81	0.59	0.49
	3,000	31.94	10.75	5.46	3.69	2.81		3,000	16.05	5.46	2.81	1.93	1.49
	5,000	55.94	18.75	9.46	6.36	4.81		5,000	28.05	9.46	4.81	3.26	2.49
25A	400	1.02	0.45	0.31	**0.26**	**0.23**	25A	400	0.59	0.31	**0.23**	**0.21**	**0.20**
	1,000	8.22	2.85	1.51	1.06	0.83		1,000	4.19	1.51	0.83	0.61	0.50
	3,000	32.22	10.85	5.51	3.72	2.83		3,000	16.19	5.51	2.83	1.94	1.50
	5,000	56.22	18.85	9.51	6.39	4.83		5,000	28.19	9.51	4.83	3.28	2.50
32A	400	1.24	0.52	0.34	0.28	**0.25**	32A	400	0.70	0.34	**0.25**	**0.22**	**0.21**
	1,000	8.44	2.92	1.54	1.08	0.85		1,000	4.30	1.54	0.85	0.62	0.51
	3,000	32.44	10.92	5.54	3.75	2.85		3,000	16.30	5.54	2.85	1.96	1.51
	5,000	56.44	18.92	9.54	6.42	4.85		5,000	28.30	9.54	4.85	3.29	2.51

LOCAL POWER SUPPLY FOR TYPICAL CONSUMERS

Consumer profiles described before are the basis for choosing an appropriate power supply for the typical consumers. The profiles were set for the situation, where the relatively cheap electricity from the grid is consumed. When using a local grid, it should be noted that price for 1 kWh is several times higher than in case of the traditional grid. Due to this, consumer profile usually changes when the transmission to local grid is made.

Sadly, as the investigation is made in Estonia, there are not so many local grid users and a systematic research on their consumption has not been followed through. It is clear however, that finding them in a new situation, consumers try to choose devices with lower power consumption (e.g. LED-lights instead of traditional bulbs) and try to avoid using electricity for heating. In a similar way the consumers try to avoid useless electricity consumption (e.g. using lighting for no apparent reason). Taken this into account, it can be assumed that in case on local grid the energy consumption can decrease up to two times compared to traditional grid consumption.

To choose the needed devices for local grid, a selection of devices available freely in the market has been made. Devices still in the development process have been discarded in this investigation. The chosen power generation devices are as follows: wind generator; PV panel; diesel generator and battery.

In the case of similar price, devices with less need of maintenance and unpleasant side-effects are preferred during the choosing of the generation devices. The device with the lowest maintenance need is the PV panel, followed by batteries, wind generator and eventually diesel generator, which is also a source for loud noise.

In every case, battery is the essential part of the local power system as the energy production of wind generators and PV panels is unsteady in time and the noise of the generator might become disturbing during night hours. In addition to that, the resource of the generator would be used up too quickly when kept working constantly. Price of the battery is mainly set by its energy capacity – the smaller the capacity the lower the price.

The time when energy supply is coming solely from the battery is called battery autonomous time. When the local power supply system contains a diesel generator, the suggested battery autonomous time is two days. If the generator is left out of the system, the suggested autonomous time rises to four days. In some cases, it is wise to optimize the battery autonomous time in relation to total cost of the system or price of the kWh.

If the system contains no generator, it is not worth to pursue 100% supply reliability from the system. This would simply make the battery too large in dimensions and of course more expensive. In rear occasions (a few percent of the time) one should accept the lack of electricity or limited usage of it (e.g. emergency lighting only). When the system has a generator installed, such limitations are generally not necessary, but in case of generator down time there is the chance of exhausting the batteries and losing the possibility of electricity consumption.

There are two main renewable energy generation units that can be utilized in a local power supply grid. These are either a PV panel, or a wind generator. The biggest drawback in case of PV panels is the inconsiderable amount of energy production during December and January in countries sharing the latitude with Estonia. In some weeks during those two months the energy production can decrease to zero.

Wind generator is not suitable for places, where the annual average wind speed is under 5 m s^{-1}. In such cases the needed power of the wind generator will grow too high, which makes the generator economically not feasible. In case of Estonia, annual average wind speed on the coast and islands at 10 m high exceeds 5 m s^{-1} (Fig. 4 usual high for small households and summer cottages). Inland however, there are many places where the 5 m s^{-1} margin is not reached. In the places where the winds are not sufficient, yet supply reliability is needed, a combination of PV panel and diesel generator should be preferred. The usage of PV panel and wind generator together will level the energy production in time, but will also make the system more complicated and expensive.

Anyway, the usage of battery would still be essential, as silent nights with no wind are not rare. Thus it is reasonable to use a system with just one renewable energy source – either PV panel or wind generator.

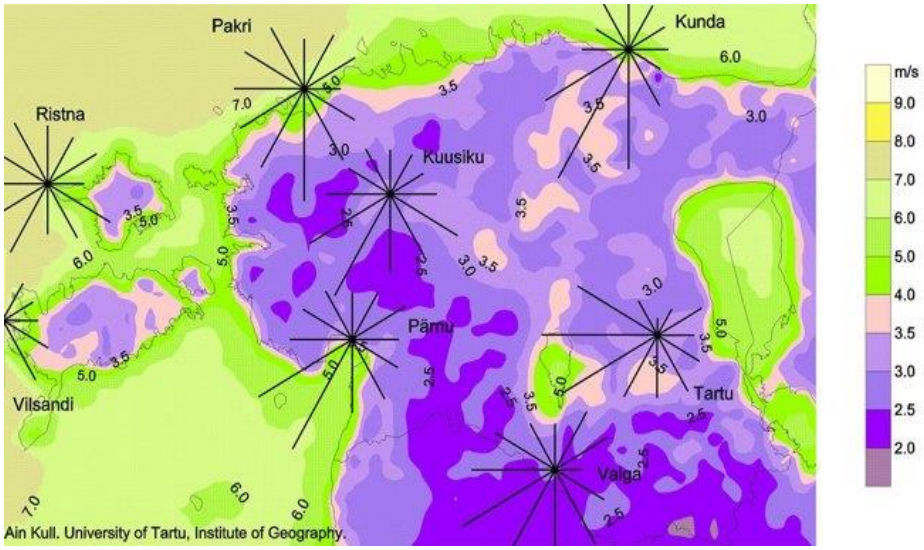

Figure 4. Annual average wind distribution in Estonia at 10 m high (Kull, 1995).

Usage of wind generator and batteries would in principle grant a reliable electricity supply. It should be investigated however, what should be the optimal battery autonomous time. It would also be good to know, if adding a diesel generator to the system would lower the price for the whole system, as diesel generators lowers the needed energy capacity of the batteries and thus the price, which is normally relatively high.

Taking all of this into account it would be reasonable to assemble the local power supply grid with the following energy sources:

1. PV panel and battery for the summer house (it is assumed that the house is not used during winter months);

2. PV panel, diesel generator and battery or wind generator and battery for the places in need of reliable supply with winds less than 5 m s^{-1}.

Table 4. Electricity prices of local power supply grids

Consumer	Elements of local grid	Annual consumption (kWh)	Price of electricity (€ kWh^{-1})
Summer house	PV panels (+ inverter), batteries	326	0.87
Cottage 1	PV panels (+ inverter), diesel generator, batteries	3,650	0.93
Cottage 2	Wind generator (+ inverter), diesel generator, batteries	3,650	0.96

Complete choosing process of the elements of local power supply grid is not presented in this paper. Results of the analysis are shown on Table 4. Those prices can now be compared to the prices of building new distribution lines shown in Table 3, where traditional power supply grid alternatives that are economically more effective are shown in bold. Other possibilities that suggest cheaper prices for local grids are shown in regular font.

CONCLUSIONS

If the case with a one family household living in a cottage is observed, it can be said that building new distribution lines is economically feasible, when the life span of the object is at least 20 years and the distance to the nearest 10 kV line is less than 800 m. With object life span of 30 years, the distance to the 10 kV line will grow to 1,000 m. In any longer distances building a local grid would be the reasonable choice.

There is an ecologically friendly and economically feasible alternative to traditional construction of substations and distribution lines, which is the autonomous local grids which are generating electricity from renewable energy sources. The solution is always applicable in the case of low consumption (up to 1,400 kWh y^{-1}) and long distances to nearest MV lines.

It can be concluded that in the case of small consumption, such as the summer house, local grid based on PV panels is always a more cost effective solution if compared to the building of new distribution line and substation even in the latitudes as north as Estonia. As studies show, the usage of wind generators on the coast or islands of Estonia with batteries energy storage system would also grant a reliable electricity supply that can be located far away from the substations.

Further study in the topic is needed to take into account the rising electricity prices due to open markets and rising prices of traditional power plant fuels. On the other hand, more accurate modelling of local grids and optimization using more precise data is needed to evaluate the prices of similar off-grid hybrid power supply systems.

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The impact of differently solved machine lines and work procedures of feeding and bedding on dust concentration in stables for dairy cows

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Abstract. The measurements were aimed at the impact of differently solved feeding and bedding systems on dust concentration in stables for dairy cows. Dust particles can be found in the environment in which the animals are housed and can affect their welfare and health, and can also negatively affect equipment in livestock buildings. Measurements of dust aerosol on 7 different farms for dairy cows in 9 stables in total were carried out. We focused on the impact of differently solved machine lines and work procedures of feeding and bedding, especially feeding with a mixer feeder wagon, bedding with a bedding wagon (equipped with a floor conveyor, a milling cylinder and a transversal conveyor), bedding with a straw blower, laying of deep litter with a wagon equipped with a hydraulic arm and manual distribution. Technical systems are especially affected by the presence of dust particles larger than 10 μm, which fall down very quickly and easily settle on the animals and surfaces in the stable. So, we measured the concentration of dust aerosol with an aerosol monitor by means of a 10 μm filter.
The results of the measurements show that feeding with a mixer feeder wagon increases the concentration of dust particles in the stables by the least amount. Straw bedding increases the concentration of dust particles in the stables by several times. This increase, however, is short in duration, and dustiness in the stables quickly returns to the state before bedding. There is a clear, substantive difference between the differently solved bedding systems.

Key words: dairy farm, feeding and bedding operations, dust concentration.

INTRODUCTION

The aim of the measurements was to determine the impact of the most commonly used systems of feeding and bedding on the concentration of dust aerosol in a stable environment. To determine the effect of feeding and bedding on the concentration of dust aerosol in a stable environment, it is important to observe changes in the dust concentration before commencing work operations, concentration changes during work operations, and how quickly the concentration of dust aerosol returns to the state before commencing work operations.

Dust settles down on most building surfaces and may lead to a deterioration of building materials and equipment in buildings for livestock. Grains of sand can penetrate into electronic devices and settle down on the temperature and humidity sensors of climate control systems. Dust can also settle down on fan motors and may result in overheating. A study of corrosion in heat exchangers used in animal stables showed that

water condensate and dust on the heat exchanger surfaces enabled microorganisms to grow and created a corrosive microenvironment.

Dust in the ventilation stream in livestock stables has been implicated in transporting and even magnifying odour. Odorous molecules (odorants) are absorbed on particle surfaces and then desorbed in large local concentrations in the nasal epithelium, where the olfactory nerve cells are located. Dust in pig houses contains volatile fatty acids (VFA),which are assumed to be indicator compounds for odour (Takai et al., 1998).

For comparison, the U.S. Environmental Protection Agency's (US EPA, 2006) National Ambient Air Quality Standards (NAAQS) limit primary and secondary dust concentrations (PM_{10}) for a 24-hour average sampling period to 150 μg m^{-3}. The purpose of the primary standard is the protection of public health, and the purpose of the secondary standard is to protect the public from known or anticipated adverse effects.

Cathomas et al. (2002) mention that the particles greater than 10 μg (PM_{10}) represent on average approximately 63% of all dust particles in the atmosphere of stables in the Alpine region and that the amount of dust particles varies throughout the year. This amount also depends on the type of construction of the stable. The measured values of airborne dust PM_{10} ranged between 76–4,862 μg m^{-3}, while the higher values were recorded in the summer period.

Jílek et al. (1998) mention that the movement of dust particles in the atmosphere depends on their size, while very small particles don´t settle down at all. The rate of sedimentation for larger particles depends on their size. From the viewpoint of the impact on the health of livestock, the less harmful particles are those exceeding 10 μm, which are captured in the nasal cavity (Jílek et al., 1998). Smaller particles are inhaled. While particles 5–10 μm are captured in the upper part of the respiratory tract, smaller particles can readily penetrate into the lungs (Dolejš et al., 2005).

The dust particles are deposited on stable equipment and reared livestock. These particles represent a breeding ground for various microorganisms and molds. Emission limits are specified for the permissible concentrations of dust in the air. Under these emission limits, we understand the maximum permissible amount of a pollutant emitted into the atmosphere from a pollution source is expressed as a mass concentration of a pollutant or a mass flow of a pollutant per unit of time or as the mass of a pollutant per unit of production or human activity. The emission limit designed for the protection of human health is determined for particles that pass through a size-selective input filter, which has, using an aerodynamic diameter of 10 μm, a separation efficiency of PM_{10}.

For flue dust PM_{10}, a 24-hour limit of 50 μg m^{-3} is applied, and this limit may be exceeded up to 35 times annually. Another limit in force determines the highest average concentration for the whole year to be 40 μg m^{-3} (Czech Republic, 2012).

For employees, the Permissible Exposure Limit (PEL) for 8 working hours in the case of dust with predominantly non-specific effects, such as soil dust, is set at 10 mg m^{-3}. In the case of dust with a predominantly irritable effect, such as cereal dust, the PEL is determined to be 6 mg m^{-3} (Czech Republic, 2007).

From the viewpoint of livestock breeding, Jílek et al. (1998) state that the maximum permissible dust content in stable air should be 6–10 mg m^{-3}.

MATERIALS AND METHODS

In Central Bohemia on 7 different farms for dairy cows in 9 stables in total measurements of dust aerosol during bedding and feeding operations were carried out.

We focused on the basic descriptions of stables and used technologies in Table 1. All of the stables were free stall designs, but they differed in the type of housing, bedding materials, and used technology. In all the stables, TMR (total mixed ration) is used for feeding cows, except dry (non milk producing) cows on Farm 1, Stable B, where forage is used. We focused on the impact of differently solved machine lines and work procedures of feeding and bedding operations, especially feeding with a mixer feeder wagon, bedding with a bedding wagon (using a floor conveyor, a milling cylinder and a transversal conveyor), bedding with a straw blower, laying of deep litter with a wagon using a hydraulic arm grapple and manual distribution.

Table 1. Description of measured technologies

Measurement	Housing *	Bedding	Work operation	Machine
Farm 1 Stable A	6-rowfreestall stable, volume 53 m^3 cow^{-1}	dried manure solids	feeding	Faresin Leader 1400 self-propelled feeding wagon
Farm 2	4-row free stall stable, volume 45 m^3 cow^{-1}	rubber mats	feeding	Zetor 6911, Silo-King Duo mixer feeder
Farm 3	4-row free stall stable, volume 45 m^3 cow^{-1}	chopped wheat straw	bedding	Zetor 5211, Kamzík Mini feeding and bedding wagon
Farm 4	3-row free stall stable, volume 62 m^3 cow^{-1}	chopped wheat straw	bedding	Zetor 6911, ZP 5-005.1feeding and bedding wagon
Farm 5	4-row free stall stable with calving pens, volume 48 m^3 cow^{-1}	chopped wheat straw	bedding	John Deere 5090M, ZP 5-005.1feeding and bedding wagon
Farm 6 Stable A	4-row free stall stables, volume 45 m^3 cow^{-1}	chopped wheat straw	bedding	Zetor 6911, obsolete bedding wagon discharging in the front
Farm 6 Stable B	4-row free stall stable, volume 45 m^3 cow^{-1}	chopped wheat straw	bedding	Zetor 6911, obsolete bedding wagon with discharging on the side
Farm 1 Stable B	shelter shed for dry cows, volume 55 m^3 cow^{-1}	chopped wheat straw (deep bedding)	bedding	Zetor 7015, wagon with discharging hydraulic arm grapple
Farm 7	2-row free stall stable, volume 35 m^3 cow^{-1}	chopped wheat straw	bedding	Zetor 7211, Romet straw blower

* All stables are naturally ventilated.

We measured the concentration of dust aerosol (fraction of PM_{10}) with a DustTrak 8520 aerosol monitor with a 10 μm filter inside the stable. Simultaneously, microclimate conditions were indicatively measured with an Almemo 2290-4 multichannel data logger with a FHA646-E1 temperature and humidity sensor and a FVA915-SMA1 wind speed

sensor. In each stable, we carried out two measurements and used these to calculate the average values.

The aerosol monitor was placed in the middle of the feed passage 1m above the floor and at least 3 m away from the walls and dairy cows.

Before work operations commenced, we measured the concentration of dust aerosol in the background c_b for 15 minutes. During feeding and bedding, we tracked the concentration c_o during the whole operation and then tracked it back to the concentration level prior to the state before commencing work operations and for at least 15 minutes thereafter.

RESULTS AND DISCUSSION

The microclimate conditions of the measurements are shown in Table 2. In all the stables, a system of natural ventilation is used, and the wind speeds are highly variable. For this reason, the measured average wind speed shown is only indicative.

Table 2. Microclimate conditions of measurements

Measurement	t_e °C	RH_e %	w_e m s^{-1}	t_i °C	RH_i %	w_i m s^{-1}
Farm 1, Stable A	12.2	68.5	2.4	16.3	61.4	3.1
Farm 2	18.4	48.2	0.8	17.0	53.0	0.4
Farm 3	8.5	95.3	3.3	10.2	92.4	2.7
Farm 4	20.4	50.8	1.2	18.6	61.2	0.4
Farm 5	3.3	86.6	0.5	4.6	92.1	1.7
Farm 6, Stable A	20.3	39.4	2.4	20.5	42.9	4.5
Farm 6, Stable B	20.6	39.2	2.7	19.0	54.0	4.0
Farm 1, Stable B	12.4	64.1	2.3	13.1	62.8	2.1
Farm 7	17.5	77.2	0.3	20.5	72.7	0.5

t_e – outdoor temperature, RH_e– outdoor relative humidity, w_e – outdoor wind speed, t_i – indoor temperature, RH_i – indoor relative humidity, w_i – indoor wind speed.

The concentration of dust aerosol in dairy stables depends on the type of work operation and the machines used. The results of the measurements are summarized in Table 3.

Table 3. Measured concentrations of dust aerosol PM_{10} during work operations

Measurement	Work operation	$c_{b\,avg}$ mg m^{-3}	$c_{b\,max}$ mg m^{-3}	$c_{o\,avg}$ * mg m^{-3}	$c_{o\,max}$ mg m^{-3}
Farm 1, Stable A	feeding	0.031	0.692	0.072	2.345
Farm 2	feeding	0.037	0.046	0.066	0.555
Farm 3	bedding	0.152	0.554	2.810	15.740
Farm 4	bedding	0.046	0.693	16.762	81.620
Farm 5	bedding	0.072	1.128	4.131	20.005
Farm 6, Stable A	bedding	0.026	0.867	2.950	24.503
Farm 6, Stable B	bedding	0.031	0.931	2.344	12.837
Farm 1, Stable B	bedding	0.068	1.492	0.198	3.439
Farm 7	bedding	0.082	0.260	4.235	10.033

* c_{oavg} – maximum of 1-minute average.

The average concentration of dust aerosol in the background $c_{b\,avg}$ varied from 26 to 152 μg m^{-3}. Almost all the values were below the 24-hour limits of the NAAQS150 μg m^{-3} (US EPA, 2006) standard, and more than half of them were lower than the 24-hour limit of 50 μg m^{-3} (Czech Republic, 2012). The maximum concentration of dust aerosol in the background $c_{b\,max}$ varied from 46 μg m^{-3} (shelter shed) to 1.128 mg m^{-3}.

The technical systems used significantly affected the presence of dust particles larger than 10 μm, which fall down very quickly and easily and settle down on animals and surfaces in the stable. In order to determine how the machine lines affect the concentration of dust aerosol, we used the absolute maximum of concentration $c_{o\,max}$ and the maximum of the 1-minute average $c_{o\,avg}$, which is significantly lower than the absolute maximum. The difference between these values shows how quickly dust particles settle down.

During feeding operations, the maximum concentration of dust aerosol $c_{o\,max}$ was higher when using a self-propelled feeding wagon (2.345 mg m^{-3}) than when using a Zetor 6911 tractor with a semi-trailer mixer feeder (0.555 mg m^{-3}). The maximum of the 1-minute averages during feeding operations were almost equal at 0.066 and 0.072 mg m^{-3}, respectively.

Bedding with straw increases the concentration of dust particles in the stables by several times. This increase, however, is short, and dustiness in the stables quickly returns to the state before bedding.

The lowest concentration during the bedding operation was achieved when using a hydraulic arm grapple in the shelter shed for dry cows. The absolute maximum concentration was 3.439 mg m^{-3}, and the maximum of the 1-minute average was only 0.198 mg m^{-3}.

The maximum of the 1-minute average concentration of dust aerosol during bedding operations using bedding wagons and straw blower was 2.344–4.235 mg m^{-3}, except for Farm 4 where the bedding material was apparently very dry and dusty and the measured concentration exceeded 16 mg m^{-3}.

An indicative measurement of microclimate conditions, in accordance with the methodology used by Fabianová et al. (2014), didn't show any effect on the measured data.

All measured concentrations of dust aerosol are in accordance with the dairy stable environment recommendations of Jílek et al. (1998). Except for Farm 4, however, the excess concentrations were short in duration. All measured concentrations are also in accordance with the given 8-hour PEL in the workplace in the Czech Republic (2007).

In the graphs in Figs 1 and 2, an example of the measured concentration of dust aerosol during straw bedding operations, using a straw blower and wagon with a discharging transverse conveyor, is shown. The maximum of the 1-minute average concentration was higher during bedding operations using a straw blower. However, the straw blower passed through the stable more quickly, and the dust particles settled down within a short time.

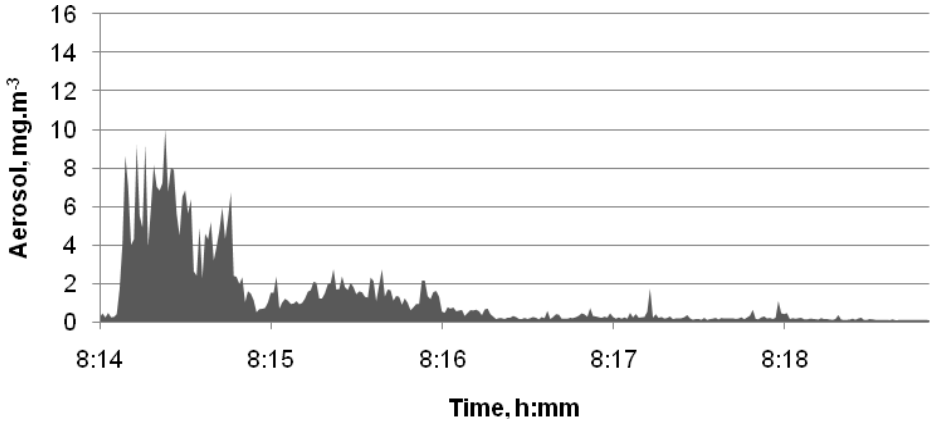

Figure 1. Concentration of dust aerosol during the laying of straw bedding with a straw blower (Farm 7), PM_{10}.

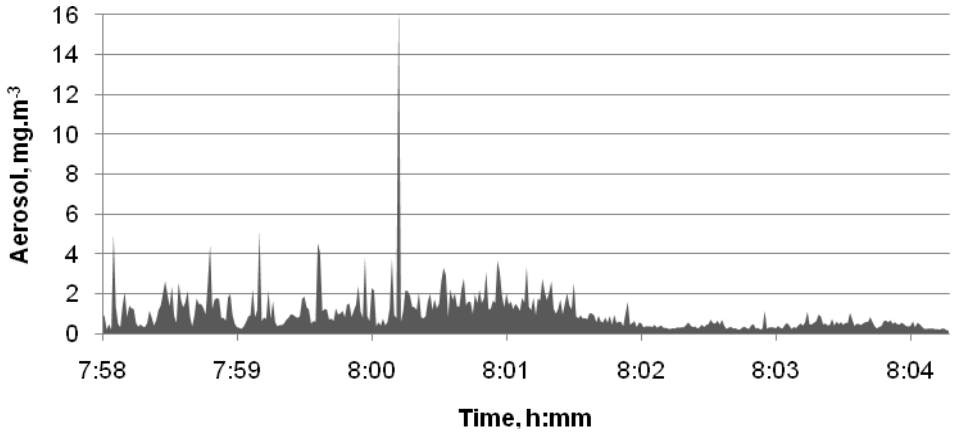

Figure 2. Concentration of dust aerosol during the laying of straw bedding with a wagon using a discharging transverse conveyor (Farm 3), PM_{10}.

CONCLUSIONS

We observed that differently solved systems of feeding and bedding have a significant impact on the concentration of dust aerosol in animal stables, except for feeding with a mixer feeder wagon which does not significantly increase dust concentration in animal stables. By contrast, bedding with chopped straw increases the concentration of dust particles in the stables by several times. This increase, however, is short in duration, and the dust concentration in the stables quickly returns to the state before the bedding operation.

There is a difference between the differently solved bedding systems. The lowest concentration was measured in naturally well-ventilated shelter shed for dry cows where bedding operations used a wagon with a discharging hydraulic arm grapple. The highest concentration was measured in a 3-row free stall stable where bedding operations used

a ZP 5-005.1 feeding and bedding wagon with discharging on the side, where a very dry straw seemed to be used.

Based on our findings, we recommend further research to very precisely identify how bedding operations using a straw blower affect dustiness in a stable, because a low concentration of dust particles was observed, contrary to our expectations.

Increased attention should be paid to the quality of the bedding straw, because there is a reasonable suspicion that, in particular, the material of bedding, the cutting length of straw, and its physical and mechanical characteristics can significantly influence the concentration of dust aerosol in a stable during bedding operations.

These results contribute to a better understanding of how the systems of feeding and bedding influence dust concentration in animal stables, which is a significant parameter for the welfare of livestock.

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Electromagnetic fields' exposure to head, torso and limbs in office workplaces

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Abstract. The aim of this research was to investigate the electromagnetic fields in the modern office environment. Both low frequency and the high frequency electromagnetic fields were studied. The sources of elevated electromagnetic fields and the conditions under which they occur were identified. Measurements were performed by following a 14-point human body model, which characterizes the overall exposure of the sitting person.
The measurements analysis revealed the most typical sources of exposure to be loosely spread power wires and extension cables, but also power cables close to the worker's body on the floor or beneath the table. Standard office devices were also rising the exposure levels when situated in close proximity to the worker.

Key words: electromagnetic fields, radiofrequency, extremely low frequency, occupational exposure, office.

INTRODUCTION

Contemporary workplaces are increasingly being equipped with different technologies, because they help to optimize the workflow of the organization. At the same time as the number of electric and electronic appliances increases, the electromagnetic fields (EMFs) accompanied by such devices also show an increase in both the amplitude and frequencies. Wherever the electrical equipment is used it produces electromagnetic fields in some extent.

From the perspective of occupational health and safety, the spectrum of the electromagnetic radiation is broad – ranging from static fields to microwaves. By the health effects classification, the electromagnetic spectrum can be divided into:

- Static fields 0 Hz – 1 Hz;
- Low frequency fields: 1 Hz– 10 MHz;

– High frequency fields: 100 kHz – 300 GHz and 6–300 GHz. In the intermediate frequency range from 100 kHz to 10 MHz the effects are combination of low frequency fields and high frequency fields (European Commission, 2015).

The electromagnetic fields could pose a risk to both on human well-being and to society in general. Studies have shown that an adverse health effect of chronic exposure can be expressed at low levels of exposure.

EMF effects on the body may vary depending on the frequency. Experiments on animals have shown that low frequency magnetic field affects chemical and physiological changes in cells (Knave, 1992; Eglite, 2000; Rosenstock et al., 2004). Some researchers have shown that chronic exposure to weak EMFs (up to 1mT) affects the immune system, depriving body's defense capabilities (Nakagawa, 1997; Adey, 1988). Another possible EMF impact mechanism can be connected with changes at the genetic level (Goodman et al., 1989). Studies in UK have indicated that long-term exposure to power frequencies with the average level of 0.4 μT double the risk in development of leukemia for children below 15 years (Coghill et al., 1996; Binhi, 2002).

Some studies derived data about RF field effects on reproductive functions, effect of weight reducing to the newborn, RF field caused premature birth and congenital abnormalities, however other researches do not confirm such data. (Persson, 1989; Cohen, 1990; Knave et al., 1994; Artamonova et al., 1996).

There have been examples of the adverse effects on the reproductive system. Also, observed increase of body temperature, (Persson, 1989; Cohen, 1990; Knave et al., 1994; Artamonova et al., 1996).

Starting from the July 1st 2016 in all European Union member states the national legislation on the protection of workers from the electromagnetic fields must be implemented following the directive 2013/35/EU (The minimum health and safety requirements regarding the exposure of workers to the risks arising from physical agents – electromagnetic fields, hereinafter – the directive). The directive's specified limit and action levels (ALs) don't grant workers protection from long-term exposure to EMFs (Official Journal of the EU, 2013). Provisions mentioned in this Directive don't protect workers from low level long term effects, which, as suggested by some studies, may have an adverse effect in case of chronic exposure. Therefore, employers should follow best practices as common in occupational safety, i.e. reduce the risks to the minimum. The so-called precautionary principle prescribes the level of protection where safety at satisfactory level is guaranteed, i.e. risk factor does not cause illness or health abnormalities, neither in short-term or long-term. That requires different risk prevention measures, because each frequency band propagates differently and has different effects. Also, numerous electromagnetic field sources can generate a number of field types such as both low-frequency fields and the radio-frequency radiation. In the process of risk assessment it is therefore necessary to identify each electromagnetic field source and the frequency band. In risk prevention measures, each workplace must be assessed individually, as to take into account not only the EMF source characteristics, but also specifics of the workplace.

As humans can't perceive electromagnetic fields, often employees do not know if they are exposed to strong levels of EMFs or not. As discussed earlier, one of the important risk mitigation measures is workers training and so that people know under which circumstances they may get exposed to high levels of EMFs. The worker, if knowledgeable about safe working methods and EMF related risks, are often capable of

organizing their working environment and processes in a risk minimizing way. Often risk associated with the electromagnetic fields can be reduced by very simple methods and techniques (Vilcane, 2015).

The aim of this study is to determine the electromagnetic fields' levels at office workplaces and to discriminate these based on the body region (head, torso, limbs).

MATERIALS AND METHODS

In this study electromagnetic fields at computer equipped offices were investigated. The office workstations consisted usually of a desktop or laptop PC, with nearby peripheral devices (i.e. printers, scanners). There were also other office equipment, such as desk lamps, telephones, extension cords and sometimes lock-boxes, server cabinets i.e. are also encountered.

In this study we used instruments from Gigahertz Solutions: 1) HF59B radiofrequency analyzer, connected to a directional antenna HF800V2500LPE174 (Germany) and 2) low-medium frequency analyzer NFA400 from the same manufacturer. The high frequency meter measured frequencies from 800 to 2,500 MHz whereas the low frequency meter measured from 50 Hz to 400 kHz. High frequency (HF) readings were taken in RMS (root mean square) mode.

The measurements followed Koppel's 14-point model (Figs 1 & 2). For each workplace 14 readings were taken for each of the three field types: measurement rounds were taken for 1) extremely low to intermediate frequency electric field, 2) extremely low to intermediate frequency magnetic field and 3) radiofrequency field. 14-point model gives a comprehensive view of the exposure situation across the workers body. For each of the 14 points the area where the worker could be was scanned with the meter and the highest reading recorded. The measurements were taken where the human body can be situated in the workplace. The results indicate the maximum possible exposure level that the worker could be exposed to. The measurements indicate the resultant field (Koppel & Tint, 2014).

It was also attempted to identify the source for elevated electromagnetic fields, when encountered. This was done by switching on and off electrical appliances, till the field level was reduced.

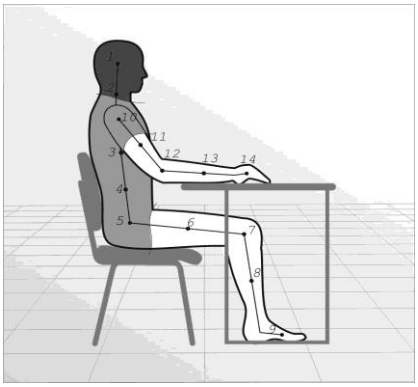


Figure 1. 14-point model of a sitting person (Koppel & Tint, 2014).

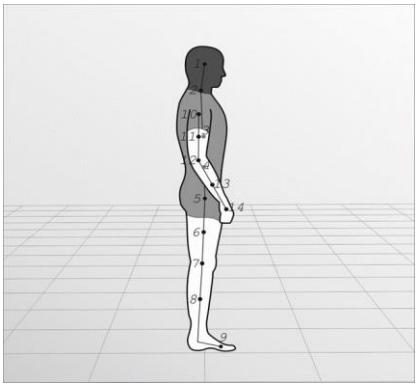


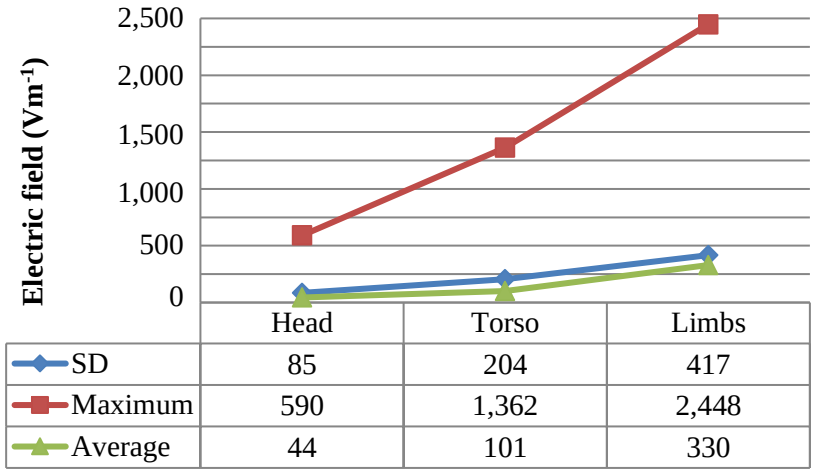
Figure 2. 14-point model of a standing person (Koppel & Tint, 2014).

For each workplace, the results from 14 points were averaged differentiating body regions into three: head (measurement points 1–2), torso (measurement points 2–5) and limbs (measurement points 6–9 and 10–14 points). Based on all the workplaces, three subsamples were formed, which characterize the exposure levels in offices (Koppel & Tint, 2014).

RESULTS AND DISCUSSION

The study covered 85 office workplaces; most of them were computer work stations. Workplaces were equipped with typical office equipment: printers, copiers, various external hard drives, stationary and radio phones, local lighting lamps, and other potential sources of exposure to electromagnetic fields, depending on the specifics of job content and the company profile. Measurements were also made in atypical office workplaces, such as security guard workstations. Latter workplaces are equipped with computers, but the nature of the job also requires equipment to perform specialized duties e.g. security camera monitoring station, walkie-talkies. Consultation room measurement data was also included in this group (Vilcane, 2015).

Based on the three subsamples, the following Figes (Figs 3, 4 & 5) show EMF exposure levels in different body areas (head, torso and limbs) (Vilcane, 2015).



	Head	Torso	Limbs
SD	85	204	417
Maximum	590	1,362	2,448
Average	44	101	330

Figure 3. Electric field exposure to different body regions in office working environments (Vilcane, 2015).

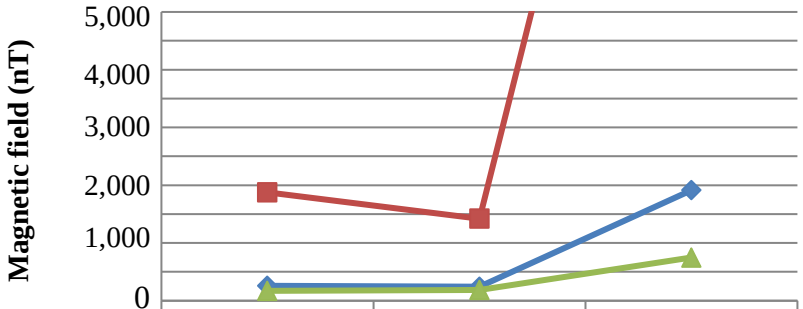
The maximum exposure case for electric field was encountered in a workplace with a badly designed electric wiring. The conductive materials in the walls and working surfaces radiated the 50 Hz electric field. In other cases, elevated electric fields were also encountered due to poor workplace layout, placement of electrical devices such as printers, computers, extension cords etc. These pheripheral devices were usually located too close to the worker's position. Comparing the average readings with standard deviation, we see that background electric field is not repetitive, however most of average readings differ from the standard deviation directly to the limbs (Vilcane, 2015).

It is important to remember that monitors and other electrical appliances should be grounded for the electric field reduction to have any effect (Sandström, 2006).

Exposure to the magnetic field to different body areas, is shown in diagram 4. From all studied workplaces, the maximum magnetic field was encountered at guardman's workplace, that was equipped with a computer and job specific appliances. The source of maximum exposure was a powerful trancievers' power supply unit. Standard deviation also showed that background exposure in surveyed workplaces, in comparison with the average reading is quite large.

Measurements also showed that portable computers' power converters also created higher than average exposure (462 nanoTeslas – nT). In latter case the exposure level was related to the distance in between the power converter and the worker's body.

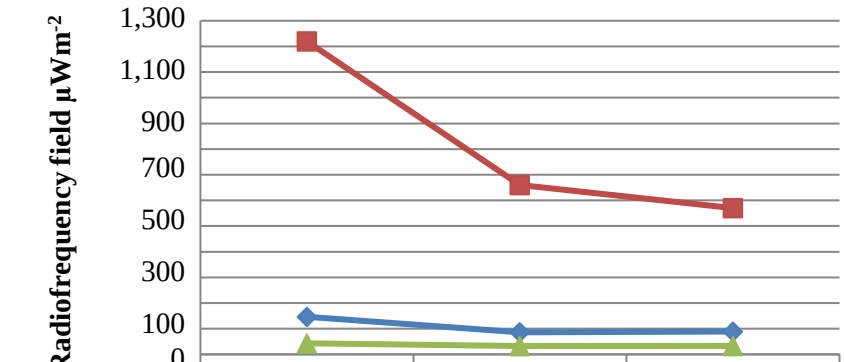
In general, elevated magnetic fields were generated by powerful office equipment – computers can generate exposure up to several thousand nanoTeslas. In one of the surveyed workplaces the largest magnetic field was from a poorly designed speaker set placed on the table (699 nT). Some workplaces are confronted with nearby transformers. In one of such workplaces, with a transformer in an adjacent room, the average background magnetic field was 491 nT across the room (Vilcane, 2015).



	Head	Torso	Limbs
SD	260	243	1,913
Maximum	1,874	1,420	16,000
Average	171	186	748

Figure 4. Magnetic field exposure to different body regions in office working environments (Vilcane, 2015).

Diagram 5 represents radiofrequency exposure in offices to various body areas. Radiofrequency radiation poses different risk specifics as compared to the low-frequency fields. If the RF radiation enters the room through the windows, generally the maximum exposure occurs in the upper body region. In this sample, mostly the RF source was from outside of the working premises, entering the room mainly through the windows (Fig. 6), (Vilcane, 2015).



	Head	Torso	Limbs
SD	147	86	88
Maximum	1,220	660	570
Average	43	33	33

Figure 5. Radio frequency exposure to different body regions in office working environments (Vilcane, 2015).

Figure 6. The source of radio frequency exposure for the highest exposure case –a cell phone base station on the adjacent building.

Often high level RF sources were cell phone base stations residing outdoors. But elevated levels were also encountered from wireless networking transmitters within the premises (Wi-Fi routers, cell phone repeaters etc), radiotelephone stations etc. In one case, a poorly selected placement for the radiotelephone network tranceiver elevated the entire office's RF levels, but was worse for the person working right half a meter away from it. The standard deviation compared with the average shows that surveyed workplace background exposure is nearly uniform.

CONCLUSIONS

This study presents results from electromagnetic fields' measurements from office environments. The measured EMF levels were below the occupational safety limits and far from levels present in some industrial processes. The aim of this article was to determine the average EMF levels at office workplaces, and to identify the sources where levels are elevated.

Based on the measurement results, there is no mandatory need for the employer to mitigate the exposure levels. However, as discussed earlier, the regulation based on the EU directive 2013/35/EU is for the protection from short-term effects, i.e. the effects from long term exposure are yet not accounted for. Some studies have shown that prolonged exposure at levels below the current safety limits may indeed have some effects (Hinrikus at al., 2005; Hardell, Sage, 2008).

This study determined that in case of extremely low frequency fields, most elevated exposure cases are due to poorly arranged electric and electronic equipment or poor workplace layout. In the majority of the cases the exposure could be reduced by rearranging the equipment and the workplace, with minimal effort from the employer's side. In general, the exposure could be reduced by creating more distance in between the worker and the equipment that is the source of the elevated levels. No conclusion can be drawn, which type of equipment should be distanced away from the worker: depending on the model, the same type of equipment could create low or high levels due to the different electrical design. Measurements are a helpful tool for the employer to determine which equipment generates high levels, so that EMF risk management plan could be implemented.

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