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Evaluation of various legume species and varieties grown in Latvia as a raw material of plant-based protein products

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Abstract. Nutrition value of legumes has been traditionally attributed to its high protein content. Protein content of legumes is variable dependent on different species and varieties, and highly affected by environmental factors. Usually protein quality is characterized by its owns amino acid profile in nutritional point of view. Therefore, the present study was conducted to determine the protein contents and amino acid profiles of pea (*Pisum sativum* L), faba beans (*Vicia faba* L) and soya (*Glycine max* L) grown in Latvia and evaluate their potential for food production. Overall results of a five-year analysis (2013–2017) showed that the protein content of peas, faba beans and soybean ranged from 20.0 to 26.1%, 26.6 to 30.5% and 35.9 to 40.9%, respectively. The corresponding values of total crude fat ranged from 0.8 to 1.2%, 0.7–1.3% and 16.6 to 19.3%, respectively. Results of study showed that the protein content of peas, faba beans and soybean was not differed by growing system ($p < 0.05$). The percentage of essential amino acids for pea, faba bean and soya were 34 to 38%, 57 to 59% and 62 to 64% respectively. The composition of pea flakes was preserved protein content of raw material. The pea flakes has high content of lysine of 10.1 g kg^{-1}, phenylalanine+thyrosine of 11.6 g kg^{-1} and the sum of essential amino acids of 66.4 g kg^{-1}. In nutritional point of view, pea flakes could be product with high-quality protein composition.

Key words: protein contents, protein quality, legume seeds.

INTRODUCTION

Legumes are considered the second most important food source after cereals, nutritionally valuable, and providing proteins with essential amino acids, complex carbohydrates, dietary fibre, unsaturated fats, vitamins and essential minerals for human diet (Rebello et al., 2014; Maphosa & Jideani, 2017). Legumes have higher protein content of 20–45% than that of most plant foods, twice the protein content of cereals. Peas and beans are on the lower side of the range with 17–30% proteins while lupins and soybeans are on the higher side with 38–45% protein (Mlynekova et al., 2014; Rebello et al., 2014; Strauta, 2017). Broad variation was stated between locations and traits for genotypes of pulses (Hawhorne, 2006; Kronberga et al., 2016). Pulses become more and more popular due to nutritional value as protein source for food and feed and due to its agricultural service crop properties (Hawhorne, 2006; Lepse et al., 2016; Kirse, 2017).

Furthermore, legumes are gluten free, making them suitable for consumption by celiac disease patients and could be a base for the development of many functional foods to promote human health (Maphosa & Jideani, 2017).

Following global trends in plant-based protein in the diet, there is a growing demand for legume products worldwide[1]. The global legumes market was valued at 44.9 billion USA dollars in 2017 and is expected to reach 75.8 billion USA dollars by 2025, growing at a Compound annual growth rate of 6.77% from 2017 to 2025[2]. The trend is related to the rapid increase in the number of supporters of green and sustainable lifestyle, as well as the actualization of environmental problems through the use of meat products in the diet. More than 40 million tons of crop proteins are imported annually in European Union (EU), representing 80% of EU's crop protein consumption (Petrusán et al., 2016). Mainly, soybeans and corn are imported, while local products such as peas and faba beans are common agricultural commodities in Europe. Partly using EU policy instruments, Germany, France (in cooperation with an Interbranch Organisation), and Poland have set up national plans to support the cultivation of protein crops. Denmark, Austria, and the Netherlands have introduced initiatives to promote plant proteins (Global legumes market 2019–2023, 2019). Vegetal proteins, gluten-free, high-protein content and high-value protein are the key determinants of the value-added products of the future.

Faba beans contain numerous anti-nutritional factors, such as phytic acid, tannins and protease inhibitors, reducing the digestibility of seeds or leading to some pathological conditions (Multari et al., 2015). Raw soybean contains anti-nutrients, including phytic acid (from phytates), which binds and prevents mineral absorption, especially zinc, calcium, and magnesium in the digestive tract (Humer et al., 2015). The preparation method was a key factor governing pulse final nutritional composition, depending on pulse species. Canning led to a greater decrease in proteins, total dietary fibers, magnesium or phytate contents compared to household cooking (Margier et al., 2018).

Extrusion is one of the methods of processing that may affect the nature of grain or seed components, its physical, chemical and nutritional properties (Diaz et al., 2006). It had been found to have minor effect on protein and fat content of peas, faba beans and soya (Strauta, 2017; Kudlinskiene, 2020). The extrusion has a positive effect on nutritional characteristics, because it induces important modifications on starch and proteins, enhancing their digestibility, and reduces the content of trypsin inhibitors, lectins, phytic acid, and tannins, typically present in legumes (Kudlinskiene et al., 2020; Pasqualone et al., 2020). At the same time, the extrusion technology in the food industry provides legumes with a mild taste, as well as faster and more convenient processing in the preparation of a meal. European manufacturers are choosing extrusion processing of legumes for food production, that way offers new food applications for legumes, which have not previously shown great economic importance, such as faba bean or peas (Pasqualone et al., 2020). For instance, a Latvian manufacturer produced extruded pea using local raw material - variety 'Bruno' and looking for new possibilities to offer the consumer a wider range of products with a high-quality protein composition.

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[1] https://www.researchandmarkets.com/reports/4749653/global-legumes-market-2019-2023

[2] https://www.hexaresearch.com/research-report/legumes-market

Despite many studies on legume varieties, their productivity and cultivation technologies there are few studies on the latest locally adapted varieties and the amino acid composition of legumes, their suitability for food production.

This study was conducted to collect a five-year analysis of crude protein, crude fat and amino acid compositions of different varieties of pea, faba beans and soya grown in Latvia, and to assest their potentially for food production.

MATERIALS AND METHODS

Materials

The research was conducted at Institute of Agricultural Resources and Economics**.** The material consisted of three varieties of peas (*Pisum sativum* L), namely 'Selga', 'Almara', 'Lāsma', twelve varieties of faba beans (*Vicia faba* L), namely 'Lielplatone', 'Granit', 'Fuego', 'Jogeva', 'Olga', 'Bobas', 'Laura', 'Isabella', 'Julia', 'Alexia', Boxer', 'Vertigo' and three varieties of soya(*Glycine max* L), namely 'Laulema', 'Annushka', 'Lajma' grown in conventional (C) and organic (O) fields at the Stende Research Centre (lat. 57.1412° N, long. 22.5367° E) from 2013 to 2017. Each field experiment was carried out using a block design with four replicates, plot size was 10 m^{-2}. Legumes grown in an organic farming system have not received additional fertilizer, but in conventional system was provided with additional 0–30 kg N, 50–60 kg P_2O_5 and 75–80 kg K_20 and a conventional pesticide programme applied.

Chemical analyses

After harvesting seeds were dried till 14% moisture, cleaned and sorted using round mesh sieve 50 mm. Mean samples from all (4) replications (0.5 kg) were taken for laboratory testing. Test weight, protein, fat and amino acids composition were determined.

Protein content was determined by the Kjeldahl method, and conversion factor 6.25 was used to convert total nitrogen to crude protein by a standart official method of analysis of LVS EN ISO 20483:2014. *Fat* was extracted with petroleum ether (boiling range of 40–60 °C) by the Soxhlet extraction method and determined gravimetrically by a standart official method of analysis of ISO 6492:1998. The content of *starch* was determined by a standart official method of analysis of LVS EN ISO 10520:2001.

Amino acids. Dried, defatted samples were treated with constant boiling 6N hydrochloric acid in the oven at around 110 °C for 23 h using the Waters AccQ Tag chemistry package. Hydrolyzate was diluted with 0.1% formic acid. Amino acids were detected using reversed-phase HPLC/MS (Waters Alliance 2695, Waters 3100, column XTerra MS C18 5 µm, 1×100 mm). Mobile phase (90% acetonitrile: 10% deionized water) 0.5 mL min^{-1}, column temperature at 40 °C was used. The identity and quantitative analysis of the amino acids (AA) were assessed by comparison with the retention times and peak areas of the standard amino acid mixture.

Free amino acids of extruded pea flakes were determined using GH FID method with Phenomenex Protein Hydroxylate Kit in laboratory BIOR. The sum of essential amino acids (EAA) was calculated as

$$\sum EAA = His + Tyr + Tre + Val + Met + Iso + Leu + Phe + Lys$$

Extrusion of products

The extrusion of pea was conducted in the factory of MILZU! Ltd., and extrusion process was performed using a double-screw extruder. Temperatures for extrusion process were set 78/83/98 °C, screw speed 800 rpm^{-1}. Obtained extrudats were cooked at 130 °C for 10 minutes in conventional oven to receive soft and crispy product. In the final stage of production, the extruded peas were ground to obtain pea flakes.

Data analysis.

SPSS statistical software version 20 was used for data analysis. The following analysis were carried out: Normal distribution (Kolmogorov-Smirnov), homogeneity of variance (Levene's test) and t-test to determine the differences between the means of growing system. The differences were considered statistically significant when $p < 0.05$.

RESULTS AND DISCUSSION

The results of analyzed protein, fat and amino acids of peas, faba beans and soya, grown in Latvia are summarized in Table 1.

Table 1. Mean values of phytochemical content for three legumes over two different growing systems

Species	Growing system	Protein	Fat	Total AA	EAA	EAA/AA
		Mean ± SD (%)		Mean ± SD (g kg^{-1})		(%)
Pea *Pisum sativum* L	C	25.3 ± 2.0[a]	1.0 ± 0.2	214.8 ± 26.2	79.6 ± 10.2 [d]	34–37
	O	21.9 ± 2.2 [a]	1.1 ± 0.2	204.1 ± 13.2	77.2 ± 5.3 [d]	35–38
Faba beans *Vicia faba* L	C	28.8 ± 3.5 [b]	0.9 ± 0.3	255.6 ± 16.3	91.6 ± 5.1 [e]	57–59
	O	30.4 ± 1.1 [b]	0.9 ± 0.3	255.1 ± 17.9	93.1 ± 5.1 [e]	58–59
Soya *Glycine max* L	C	38.1 ± 1.9[c]	19.3 ± 0.7	310 ± 11.3	119.7 ± 4.4 [f]	62–63
	O	39.6 ± 1.2 [c]	18.6 ± 0.7	318 ± 9.7	122.8 ± 4.2 [f]	62–64

Different letters indicate significant differences between treatment means.

Results of Table 1 showed that the protein content of peas grown in conventional system was 25.3 ± 2.0% and it was higher than in organic system 21.9 ± 2.2% but diference is not significant ($p > 0.05$). Results of study showed that average protein content of tested varieties are similar with protein content of grey pea 26.9 ± 2.0% (Strauta et al., 2016). The average amount of essential amino acids of peas grown in conventional system was 79.6 ± 10.2 g kg^{-1} it was higher than in organic system and varied more widely over the years and varieties. The results of this study are in line with other, where the protein content of peas and beans reported 17–30% (Mlynekova et al., 2014), in dry pea grains varied between 18.8% and 33.5% depending by variety (Kronberga et al., 2016).

The protein content of faba beans determined higher than protein content of peas, it varied from 26.6% to 31.5% with mean values 28.8 ± 3.5% in samples of conventional fields and 30.4 ± 1.1% in samples of organic fields, it was not affected by growing system ($p > 0.05$). Average of total amino acids content of faba beans determined in conventional system of our study was 255.6 ± 16.3 g kg^{-1}, it was higher than reported in other studies - 232.3 g kg^{-1} (Kudlinskiene et al., 2020). The percentage of essential amino acids in total amino acids for faba bean determined 57–59%, it was higher than for peas and higher as reported for faba bean in other studies - 34–41% (Toews & Wang, 2013) or 31.8–37.7% (Alghamdi, 2009).

The protein content of soybeans determined in this study varied from 35.9% to 40.9% and fat content varied from 16.6% to 19.3%. Results of Table 1 showed that the protein content of soya beans grown in conventional system was 38.1 ± 1.9% and in organic system 39.6 ± 1.2% but diference is not significant ($p > 0.05$). Degola et al. (2019) reported that the protein content in soya bean samples determined from 32.7% to 40.7% and fat content from 18.4% to 21.4% and significantly differed ($p < 0.05$) among growing places in Latvia. The percentage of essential amino acids in total amino acids for soya bean samples was determined from 59% to 62%. The results of this study, when compared protein, fat and percentage of amino acids with values of soya beans mentioned in USDA (2018) database (36.49%, 19.94% and 63.7% respectively) shows that composition of soya grown in Latvia is equivalent in terms of protein and fat content. Grieshop & Fahey (2001) reported that soybeans from China had greather crude protein concentration (42.14%) than those from Brazil (40.86%). Environmental conditions under which soybean are grown have a great impact on chemical composition - diferences in crude protein, amino acid and lipid contents of soybeans were detected both within and among countries (Grieshop & Fahey, 2001).

Protein content of legumes is variable dependent on different species and highly affected by environmental factors. Therefore was compared protein content of species in different years, the results was showed in Table 2.

Table 2. Mean protein content for three legumes for five harvest years

Species	Protein content, mean ± SD %					Average
	2013	2014	2015	2016	2017	
Pea *Pisum sativum* L	22.8 ± 1.9	26.1 ± 2.1	24.5 ± 2.1	26.0 ± 2.0	24.5 ± 2.1	24.37 ± 2.2
Faba beans *Vicia faba* L	26.8 ± 2.1	30.1 ± 2.5	29.6 ± 1.2	24.7 ± 2.1	26.4 ± 2.1	28.0 ± 1.5
Soya *Glycine max* L	*	39.59 ± 2.5	38.1 ± 2.5	*	*	38.8 ± 2.7

*No data.

The results of this study, summarized in Table 2, showed that protein content of legumes was affected by year, the diference for pea and soya beans was significant ($p < 0.05$). Average protein content of twelve varieties of faba beans was not differed significantly, because reaction of each variety on environmental factors was genetically affected due to different ripening times. Differences in protein content of legumes by year can be explained by different amounts of precipitation and air temperature from year to year. Average daily air temperature and cumulative solar radiation during seed filling are especially important for soy ripening. Carrera et al. (2011) also concluded that the environment was the most important source of variation for all traits of soya beans, followed by the genotype x environment interaction.

To characterize potential of varieties grown in same climatic conditions the protein content of different varieties of legumes grown in Latvia was reflected in Fig. 1.

The results of protein summarized in Fig. 1 are in line with other scientific investigations where mentioned that peas and beans are on the lower side of the range with 17–20% proteins while soybeans are on the higher range with 38–45% protein (Maphosa & Jideani, 2017) with the remark that protein content of evaluated varieties was higher than 20%. The results of this five-year study showed that the highest protein content determined in samples of pea variety ‘Almara’ 26.3 ± 2.2%, but lowest protein content in variety ‘Selga’. Most bean varieties evaluated in this study demonstrated average protein content 25–26%. Varieties of faba beans with highest potential to form protein in seeds were ‘Jogeva’ with average protein content 29.9 ± 2.5%, ‘Lielplatone’ - 28.0 ± 2.7% and ‘Julia’ - 27.1 ± 2.6%.

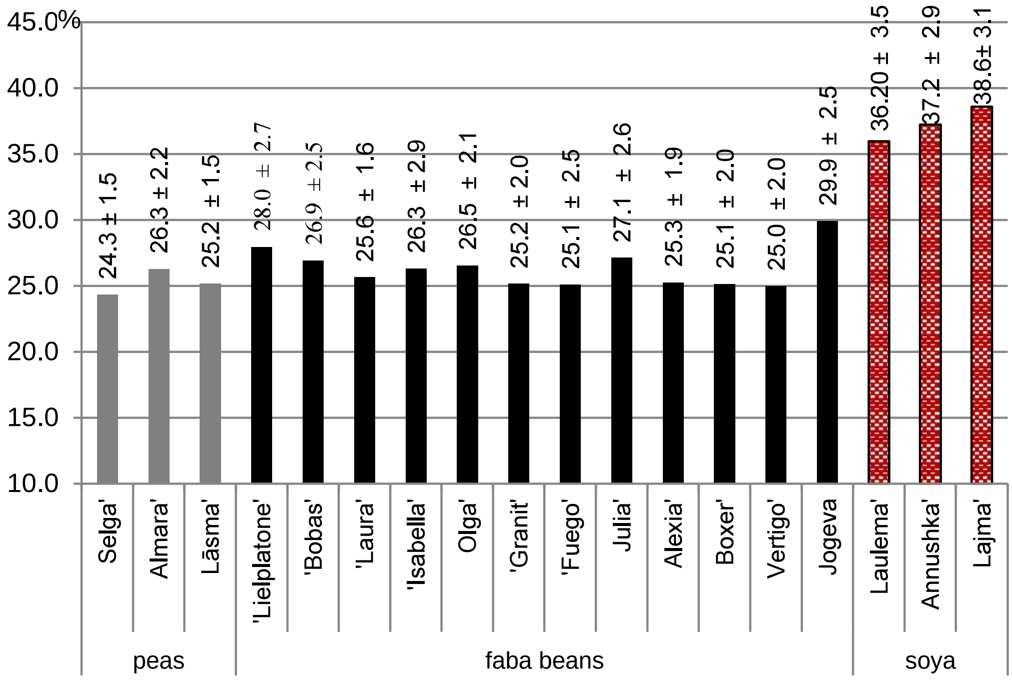

Figure 1. Comparison of protein content of pea, faba beans and soya varieties grown in Stende.

The protein content in samples of soya variety ‘Lajma’ - 38.6% determined higher than in samples of variety ‘Laulema’ - 36.2% ($p < 0.05$).

Protein content is not the main indicator of nutrition value of food, more important is composition of amino acids. The analyzed levels of essential amino acids were not always directly related to the protein concentrations of the samples (Goldflus, 2006). Comparison of essential amino acids of pea, beans and soya by years 2014 and 2015 is shown in Fig. 2.

The results of this study showed that content of all amino acids determined higher in the year 2014, especially different were content of Val, Ile, Leu, Phe an Lys in pea and faba bean samples and content of Met, Phe, His and Lys in soya samples. The predominant essential amino acids were leucine, lysine, phenylalanine and valine in all investigated legumes. The results of this study confirms that pulses are low in the essential sulphur containing amino acids - methionine and cysteine (Maphosa & Jideani, 2017; Margier et al., 2018). Fig. 2 shows that content of methionine in peas and faba beans was determined from 3.3 g kg^{-1} to 3.6 g kg^{-1} and the content of methione in soya beans varied from 7.8 g kg^{-1} to 8.2 g kg^{-1}.

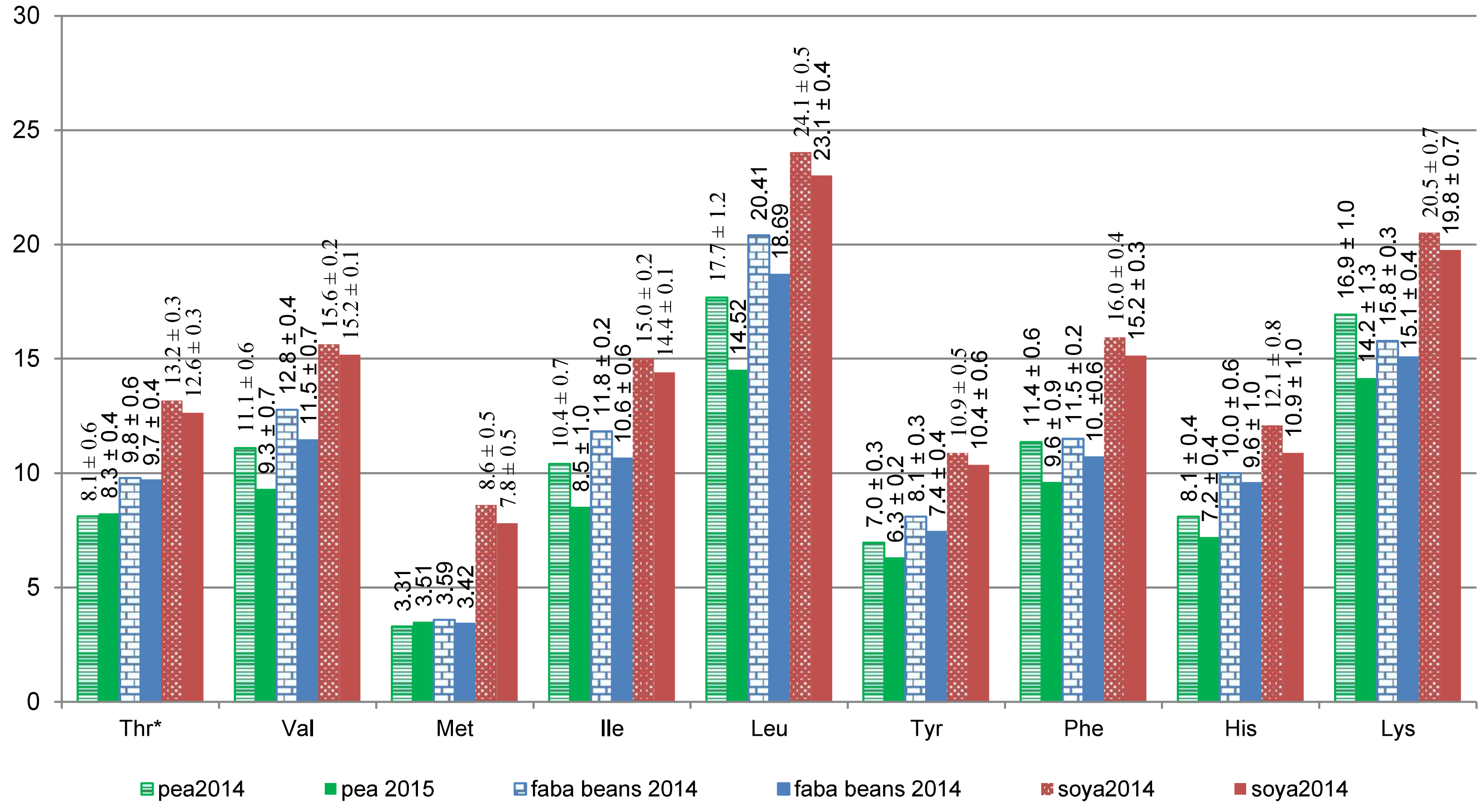

Figure 2. Comparison of essential amino acids content of pea, faba beans and soya in 2014 and 2015 years, g kg^{-1}.

The results of this study showed that content of all amino acids determined higher in The results of this study are in line with other, where evaluated amino acid composition of field beans and mentioned that content of methionine was 0.22 ± 0.01 g 100 g^{-1} content of lysine 1.65 ± 0.01 g 100 g^{-1} or content of leucine was 2.03 ± 0.01 g 100 g^{-1} (Strauta, 2017). Diaz et al. (2006) reported that, content of leucine, lysine, phenylalanine and valine in pea seeds was 14.1 g kg^{-1}; 13.0 g kg^{-1}; 9.5 g kg^{-1} and 9.1 g kg^{-1} respectively while coresponding values of amino acids in faba beans was higher - 18.8 g kg^{-1}; 15.9 g kg^{-1}; 10.9 g kg^{-1} and 12.2 g kg^{-1} respectively.

The content of methionine, phenylalanine and lysine determined in soya samples are significantly higher than content of these amino acids in pea or faba beans samples. The results of this study showed that content of methionine in soya beans was determined from 7.8 g kg^{-1} to 8.2 g kg^{-1}, phenylalanine from 15.2 g kg^{-1} to 15.9 g kg^{-1} and lysine 19.8 g kg^{-1} to 20.5 g kg^{-1}. Diaz et al. (2006) reported that the content of methionine, phenylalanine and lysine in soya meal was 6.5 g kg^{-1}, 23.5 g kg^{-1} and 28.4 g kg^{-1} respectively.

Several factors contribute to limited use of legumes - low yields, poor seed availability, anti-nutrients, their association with bloating and flatulence and their hard-to-cook feature. The development of new legume products could lead to a higher demand of legumes and to increase the production of these legumes by local farmers.

High-temperature short-time extrusion technology (HTST) has become popular in preparing breakfast cereals or snacks of starchy base products like pea and beans.). The plant protein trend has promped innovation in meat substitutes and more than two-thirds of all products included legume ingredients such as adzuki and black beans, chikpeas and lentils with products containin between 9% and 65% legume ingredients (Curtain & Grafenauer, 2019).

Extruded pea flakes for plant-based products manufacturing was prepared. The composition of untreated pea and extruded pea flakes are showed in Table 3.

Table 3. Composition and nutrition value of extruded pea flakes

Nutrients	Untreated	Extruded	Difference*
Protein, %	17.8	17.1 ± 0.2	NS
Fat, %	1.39	1.50 ± 0.1	NS
Starch, %	67.3	67.2 ± 1.5	NS
Dietary fibre, %	8.4	8.0 ± 0.5	NS

*NS – insignificant ($p > 0.05$).

The results of untreated pea and extruded pea flakes summarized in Table 3 are in line with other scientific investigations made on the extrusion of the faba beans and peas - protein and starch are not lost in the extrusion process. In the other studies about extrusion of legumes mentioned that, the protein content of untreated grey pea flour - 26.1 g 100 g^{-1} and extruded pea - 26.9 g 100 g^{-1} was not differed significantly (Strauta, 2017), but in the faba bean samples protein content decreased from 32.5 ± 0.7 g 100 g^{-1} before extrusion to 31.5 ± 0.5 g 100 g^{-1}after extrusion (Strauta & Muizniece-Brasava, 2016) or increased from 232.2 ± 2.0 g kg^{-1} before extrusion to 272.0 ± 3.1 g kg^{-1} after extrusion (Kudlinskiene et al., 2020), but differences were evaluated as insignificant.

World Health Organization recommended daily protein intake is 45 g for an average 60 kg healthy adult female, and 56 g for an average 75 kg male (WHO, 2007). The results of study showed that with 100 g of extruded pea flakes is possible provide about 1/3 of daily protein need. Vegetarians and vegans may need to eat 10–20% more protein than recommended in order to compensate for the lower digestibility of plant-based protein (Petrusan, 2016).

The nutritional value of proteins may differ substantially depending on their amino acid composition and digestibility. Comparison of essential amino acids profile of the extruded pea flakes with recommendations of World Health Organization reflected in Fig. 3.

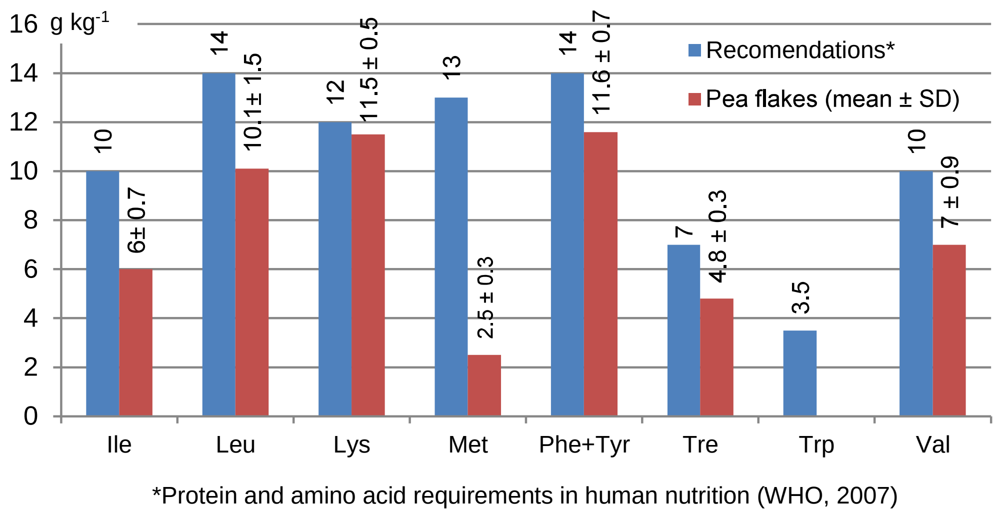

Figure 3. Composition of essential amino acids of extruded pea flakes.

The composition of essential amino acids of extruded pea flakes (Fig. 3.) showed high content of lysine - 11.5 ± 0.5 g kg^{-1}, leucine 10.1 ± 1.5 g kg^{-1} and phenylalanine 6.8 ± 1.0 g kg^{-1} its are close to recommended amount. The results of this study confirms that like other legumes pea flakes are low in the essential sulphur containing amino acid methionine. The content of methionine determined in pea flakes was 2.5 ± 0.3 g kg^{-1}. The composition of amino acids, including content of predominant amino acids lysine, leucine and also methionine of this study were in line with results for extruded grey pea reported by Strauta (2017) - 1.75 ± 0.01 g 100 g^{-1}, 1.76 ± 0.01 g 100 g^{-1} and 0.22 ± 0.01 g 100 g^{-1} respectively. Kudlinskiene et al. (2020) reported no changes in the profile of amino acids of the extruded faba beans, except for the amounts of lysine and histidine. The content of lysine decreased from 16.1 g kg^{-1} in raw material to 15.1 g kg^{-1} in the extruded faba beans, content of histidine decreased from 12.3 g kg^{-1} to 9.8 g kg^{-1} respectively (Kudlinskiene et al., 2020).

When evaluating the extruded product from the point of view of a meat substitute, must be said that protein content (17.1%) of pea flakes was lower, but the sum of essential amio acids (58.2 g kg^{-1}) and contents of lysine (11.5 ± 0.5 g kg^{-1}), leucine (10.1 ± 1.5 g kg^{-1}) or methionine (2.5 ± 0.3) were higher than in the game meat, where corresponding values were - 22.21–23.59%; 27.1–45.7 g kg^{-1}; 5.0–9.5 g kg^{-1}; 5.4–9.0 g kg^{-1}; 1.6–2.5 g kg^{-1} respectively (Strazdina et al., 2011). This comparison is only an insight that encouridges more analysis of the use of peas as a meat substitute.

Since cereals are high in sulfurcontaining amino acids and low in lysine (high in legumes) (Maphosa & Jideani, 2017) for nutritional balance, legumes and cereals are to be consumed in the ratio 35:65, because complemented each other in terms of protein.

CONCLUSIONS

The pea, faba beans and soya grown in Latvia as a raw material for food production has average protein content of five years 24.37 ± 2.2%, 28.0 ± 1.5% and 38.8 ± 2.7% respectively. No difference of protein content of legumes was observed between organic and conventional growing systems.

The percentage of essential amino acids in total amino acids for pea, faba bean and soya determined 34–38%, 57–59% and 62–64% respectively. The soya seeds grown in Latvia are suitable for high quality food production, in addition, procesors are provided with GMO free raw material. Average protein content of soya grown in Stende was 38.1 ± 2.7%, average content of essential amino acids - 122.8 ± 4.2 g kg^{-1}.

The results of extruded pea flakes confirms that protein and starch are not lost in the extrusion process, the protein content of pea flakes was 17.1 ± 0.2%. The composition of essential amino acids of extruded pea flakes showed high content of lysine - 11.5 ± 0.5 g kg^{-1}, leucine 10.1 ± 1.5 g kg^{-1} and phenylalanine 6.8 ± 1.0 g kg^{-1} its are close to recommended amount. There is possibility to produce extruded products using legumes as a raw material of local origin which could be used for high-value plant-based gluten-free protein products production.

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