Nutritional value of protein in wheat-rye bread manufactured with addition of flour from low-alkaloid cultivars of lupin

Jan Kłobukowski[1], Marzena Danowska-Oziewicz[1], Filip Kłobukowski[2], Krystyna Skibniewska[1]

1 – University of Warmia and Mazury in Olsztyn, Olsztyn, Poland
2 – Medical University of Gdańsk, Gdańsk, Poland

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Corresponding author:

Jan Kłobukowski
E-mail:
jan.klobukowski@uwm.edu.pl

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Abstract

Introduction. The aim of this study was to assess the nutritional value of protein in wheat-rye bread manufactured with an addition of flour from low-alkaloid cultivars of lupin.

Material and methods. Flours from yellow lupin cultivars: Juno, Polo, Legat and Markiz, were used in wheat-rye bread baking. The following were determined in breads: protein content, *in vitro* protein digestibility, amino acid composition, effective protein content (EP), chemical score (CS), essential amino acid index (EAAI), true digestibility of protein (TD), protein digestibility corrected amino acid score (PDCAAS) and protein efficiency ratio (PER).

Results and discussion. The addition of lupin flour resulted in an increase in the total protein and digestible protein in breads. The greatest increase in the EP, CS, PDCAAS and EAAI was observed when the bread was enriched with flour from Polo cultivar. No significant changes were noted in PER. Protein in the products with lupin flour contained more leucine, lysine, asparagine and arginine, than the cotrol sample. A tendency has been recently observed to enrich cereal products, mainly those made from wheat and wheat-rye mixes, with flours obtained from other plants, such as lupin, green peas, beans, hemp and buckwheat. These flours are highly valued because of their functional properties, which include solubility, emulsifying capabilities, foaming and gelling properties, and water retaining capability. Lupin flour and protein isolates from lupin seeds does not affect the taste of a final product. An addition of lupin flour to wheat bread improves considerably the quality and amount of protein and dietary fibre in a final product. The high values of indices PDCAAS and PER make it possible to compare lupin proteins and proteins of leguminous plants to animal proteins.

Conclusions. Protein of low-alkaloid cultivars of lupin can be a valuable component which increases the nutritional value of bread protein and therefore it should be recommended for use in baking.

Introduction

Cereal products, including bread, are at the base of many present food pyramids. They provide the human body with energy, protein of plant origin, vitamins B as well as macronutrients (magnesium, potassium, phosphorus) and micronutrients (iron, copper). Protein performs a number of functions in the body, with serving as building blocks being the most important of them. However, cereal proteins are regarded as having lower biological value than those of animal origin. This is because they contain, depending on the cereal species, smaller amounts of some essential amino acids – lysine and tryptophan (wheat and maize), methionine (rye) and threonine (rice) (Hryniewiecki, 2007).

Leguminous plants, particularly soybean, are used as a raw material in the production of food which provides the body with full-value protein. Lupin is an alternative source of protein, which can replace soybean imported from the warm climate zone. The use and consumption of products from seeds of this plant have been steadily growing in recent years (De Cortes-Sanchez *et al*., 2005). Lupin is a good source not only of protein and essential amino acids, but also of fat, dietary fibre, minerals and vitamins (Martinez-Villaluenga *et al*., 2006, 2009; Zielińska *et al*., 2008; Kohajdoova *et al.,* 2011; Schumacher *et al*., 2011). Due to a high content of lysine in lupin protein, particularly in “sweet”, low-alkaloid cultivars, it can be an excellent supplement of cereal proteins, in which the content of this amino acid is low.

In the current available literature, no data on the usefulness of the protein of bread baked from wheat-rye flour enriched with lupine flour have been found. It was decided that the nutritional value of the protein of the analyzed bread should, apart from traditional chemical quality indicators, be extended with the newest, recommended indicators taking into account the biological availability of this nutrient.

The aim of this study was to assess the nutritional value of protein in wheat-rye bread manufactured with an addition of flour from low-alkaloid cultivars of lupin.

Materials and methods

Sample preparation

Bread made from the mixture of wheat-rye (80 : 20) flours (K – control) and breads from wheat-rye flour with a 5% addition of flour from four low-alkaloid “sweet” cultivars of yellow lupin (75 : 20 : 5) were used as the study material. The experimental breads were marked after cultivars as: J – Juno, P – Polo, L – Legat, and M – Markiz. Lupin seeds were obtained from the Agricultural Experiment Unit of the University of Warmia and Mazury in Olsztyn, Poland. The bread was baked in a private bakery. The baking process was carried out as described in Skibniewska *et al.* (2003).

Analyses

The total protein (N x 6.25) was determined according to AOAC (1995a). The *in vitro* protein digestibility, i.e. its susceptibility to the action of digestive enzymes, was determined after incubation with a solution of pepsin, pancreatin and a bile extract at the enzyme-protein ratio of 1 : 20 (Ikeda, 1990). The *in vitro* protein digestibility was calculated from the following formula: ($D_{in\ vitro}$ = [a – b/a] x 100), where a – starting content of protein in the bread under study as g/100 g of bread, b – content of protein in the bread under study following *in vitro* digestion, as g/100 g of bread.

Amino acid content (g/16 g N, equivalent of g/100 g of protein) was determined with an amino acid analyser Biochrom 20 Plus (Biochrom Ltd., Cambridge, UK). The Amino Acid Standard Solution by Sigma was used as the standard. The protein hydrolysate was prepared by acidic hydrolysis (6 M HCl, at 105°C for 24 h). The content of sulphur amino acids was determined separately in 6 M HCl following oxidative hydrolysis (formic acid + hydrogen peroxide at 9 : 1 ratio, 4°C, 16 h). Tryptophan was determined according to AOAC (1995b).

The content of essential amino acids in the breads under study was used as the basis for calculation of the chemical protein quality indices: the chemical score (CS) and the integrated essential amino acid index (EAAI). The amino acid composition of two standard proteins – FAO/WHO 1991 and DRI/USA 2005 (Jarosz *et. al.*, 2017) was used in the calculations. The CS index was calculated from the formula: $X_i = a_i / a_{is}$ x 100, where a_i – the content of a given essential amino acid in the protein under test, a_{is} – the content of a given essential amino acid in the standard protein, i = 1, 2…8 (Hryniewiecki, 2007). The calculations were conducted using the content of isoleucine (ILEU), leucine (LEU), lysine (LYS), sum of methionine and cysteine (MET + CYS), phenylalanine and tyrosine (PHE + TYR), threonine (THR), tryptophan (TRY) and valine (VAL). The CS is taken as the lowest ($X_{min.}$) of the eight ratios expressed as percent ($X_{min.}$ = CS), and the amino acid for which the value is the lowest is the one limiting the nutritional value of the protein.

The EAAI, introduced by Oser (1959), was calculated from the formula:

$$EAAI = 10^{\lg EAA}.$$

In order to calculate lg EAA, the following formula, provided by Rakowska *et al.* (1978), was used:

$$\lg EAA = 1/8 (\lg X_1 + \lg X_2 + \ldots + \lg X_8).$$

The content of effective protein (EP) was calculated from the respective CS (%) and the total protein content (TP, %) in the bread under analysis, from the following formula:

EP (%) = TP x CS/100 (Gawęcki & Jeszka, 1986).

The true digestibility (TD, %) of investigated bread samples was calculated as the weighted average of the true digestibility indices of individual components of breads and their percentage share. The TD values of individual components were taken after Boye *et al.* (2012): wheat flour – 89.4%, rye flour (barley) – 75.3%, lupin flour (soybean) – 80.0%.

The protein digestibility corrected amino acid score (PDCAAS) was calculated from the formula:

PDCAAS (%) = CS (%) x TD (%)/100 (Schaafsma, 2012, Ruhterfurd 2015).

The protein efficiency ratio (PER) was calculated from the content (g/16 g N) of leucine (LEU) and tyrosine (TYR) in the bread from the following formula:

PER = – 0.468 + 0.454 LEU – 0.105 TYR (Sujak *et al.*, 2006).

The chemical analyses were performed in triplicates. The results in Table 1 and 2 are presented as mean values with standard deviations. The statistical significance of differences between the mean values was tested with the Student's t-test at the significance level of $P \le 0.05$, using the Statistica PL 10.0 software (StatSoft, Kraków, Poland).

Results and discussion

Protein content and *in vitro* protein digestibility in the breads under study

Table 1 shows the protein content and its *in vitro* digestibility in the tested samples. All breads manufactured with the addition of lupin flour contained more protein (10.1–11.0%) than the control bread (9.0%), with the bread enriched with the Markiz cultivar flour showing the highest percentage of protein. No significant differences were observed between the breads made with an addition of lupin flours. The content of protein following *in vitro* digestion was the highest in bread prepared with an addition of flour from the Juno (5.6%) cultivar, but again, no statistical differences between the enriched breads were observed. All values of this parameter were higher in the experimental samples than in the control. The protein in the bread enriched with the Juno cultivar was the most susceptible to the action of digestive enzymes (55.4%). The other digestibility indices were not statistically different and were 50.5%; 49.1% and 49.1% for breads with flours of the Polo, Legat and Markiz cultivars, respectively.

Table 1

Protein content and *in vitro* protein digestibility in the breads under study (mean ± SD)

Specification	**Type of bread**				
	Control K	**Juno J**	**Polo P**	**Legat L**	**Markiz M**
Total protein content, g/100 g	9.0±0.2^{a}	10.1±0.2^{b}	10.5±0.2^{b}	10.8±0.2^{b}	11.0±0.3^{b}
Protein content following *in vitro* digestion, g/100 g	4.4±0.1^{a}	5.6±0.1^{b}	5.3±0.3^{b}	5.3±0.1^{b}	5.3±0.3^{b}
In vitro protein digestibility, %	48.9±1.0^{a}	55.4±1.1^{b}	50.5±1.0^{a}	49.1±0.8^{a}	49.1±0.6^{a}

Different letters in a row denote the presence of differences statistically significant at $P \le 0.05$

Amino acid composition of wheat-rye bread made with an addition of selected lupin flours

Table 2 presents the content of amino acids in protein of the investigated bread samples. Modification of the dough composition significantly increased the content of leucine level from 7.4 g/16 g N in the control sample to 7.7 g/16 g N in the bread with

an addition of flour from the Legat cultivar. Nevertheless, the content of this amino acid in all investigated breads exceeds its content in the standard protein FAO/WHO 1991 (6.6 g/16 g N) and DRI/USA 2005 (5.5 g/16 g N).

Table 2

Amino acid composition of wheat-rye bread made with an addition of selected lupin flours (mean ± SD)

Specification	Type of bread				
	Control K	**Juno J**	**Polo P**	**Legat L**	**Markiz M**
Essential amino acids, g/16 g N					
Thr	2.9±0.1^{a}	3.0±0.1^{a}	2.8±0.1^{a}	2.9±0.1^{a}	2.9±0.2^{a}
Met	2.1±0.1^{a}	1.7±0.1^{b}	1.8±0.1^{ab}	1.9±0.1^{a}	1.7±0.2^{ab}
Met + Cys	3.5±0.1^{a}	3.2±0.1^{b}	3.3±0.1^{ab}	3.1±0.1^{b}	3.1±0.3^{ab}
Val	5.4±0.1^{a}	5.2±0.1^{a}	5.2±0.1^{a}	5.3±0.1^{a}	5.1±0.3^{a}
Ileu	4.2±0.1^{a}	4.3±0.1^{a}	4.1±0.1^{a}	4.3±0.1^{a}	4.2±0.1^{a}
Leu	7.4±0.1^{a}	7.5±0.1^{a}	7.4±0.1^{a}	7.7±0.1^{b}	7.6±0.3^{ab}
Phe	5.3±0.1^{a}	5.0±0.1^{b}	4.9±0.1^{b}	4.9±0.1^{b}	5.2±0.1^{a}
Phe + Tyr	7.3±0.2^{a}	7.0±0.1^{a}	6.9±0.1^{a}	7.3±0.1^{a}	7.2±0.3^{a}
Lys	2.5±0.1^{a}	3.2±0.1^{b}	3.7±0.1^{b}	2.9±0.1^{b}	3.0±0.3^{b}
Try	0.8±0.1^{a}	0.8±0.1^{a}	0.8±0.1^{a}	0.8±0.1^{a}	0.8±0.1^{a}
Non-essential amino acids, g/16 g N					
Asp	4.4±0.1^{a}	5.3±0.2^{b}	5.3±0.2^{b}	5.2±0.1^{b}	5.3±0.2^{b}
Ser	4.6±0.1^{a}	4.5±0.1^{b}	4.5±0.1^{b}	4.6±0.1^{a}	4.4±0.3^{ab}
Glu	33.0±0.1^{a}	32.1±0.1^{b}	31.8±0.1^{b}	31.5±0.5^{b}	32.0±0.2^{ab}
Pro	11.3±0.1^{a}	9.7±0.1^{b}	9.5±0.1^{b}	9.7±0.1^{b}	9.9±0.4^{b}
Ala	3.4±0.1^{a}	3.4±0.1^{a}	3.6±0.1^{b}	3.5±0.2^{a}	3.4±0.2^{a}
Gli	3.6±0.1^{a}	3.6±0.2^{a}	3.6±0.2^{a}	3.6±0.1^{a}	3.7±0.1^{a}
His	2.0±0.1^{a}	2.0±0.1^{a}	2.2±0.1^{b}	2.1±0.1^{a}	2.2±0.3^{a}
Arg	3.5±0.1^{a}	5.0±0.5^{b}	5.0±0.1^{b}	4.7±0.1^{b}	4.8±0.2^{b}

Different letters in a row denote the presence of differences statistically significant at $P \leq 0.05$

Particular improvement of the protein quality in breads with the addition of lupin flour was observed in the case of lysine. Bread with the flour from the Juno cultivar contained 3.2 g/16 g N of lysine, that with an addition of the Polo cultivar – 3.7 g/16 g N; Legat – 2.9 g/16 g N, and Markiz – 3.0 g/16 g N. The content of lysine in protein of the control sample was

2.5 g/16 g N. Regarding the non-essential amino acids, a considerable increase in the amount of asparagine was recorded in all breads enriched with lupin flour: Juno – 5.3 g/16 g N, Polo – 5.3 g/16 g N, Legat -5.2 g/16 g N and Markiz – 5.3 g/16 g N, compared to the control sample (4.4 g/16 g N). An addition of flour of the Polo cultivar increased the content of alanine in the protein from 3.4 g/16 g/N (control bread) to 3.6 g/16 g N. The content of arginine increased considerably in each option of the bread enrichment with lupin flour. It was 5.0; 5.0; 4.7; 4.8 g/16 g N for the breads with the addition of flour from the Juno, Polo, Legat and Markiz cultivars, respectively, compared to the control sample – 3.5 g/16 g N. Conversely, all breads enriched with lupin flour contained considerably less methionine + cystine, while valine and phenylalanine + tyrosine remained at the comparable level to protein in the bread made from wheat and rye flour (Table 2).

Chemical and dietary indices of the protein nutritional value of wheat-rye bread made with selected lupin flours

The chemical and dietary indices of the nutritional value of protein were calculated using two amino acid standards – FAO/WHO 1991 and DRI/USA 2005 (Table 3). The content of effective protein (EP) increased after the addition of lupin flour from each cultivar under study, and it was the highest when the bread was enriched with flour from the Polo cultivar. The values obtained for this sample with the use of both of the standards were 6.7% and 7.6%, respectively. The addition of lupin flour increased the chemical score and the essential amino acid index of all investigated samples. Lysine was the limiting amino acid in each treatment. The highest CS was calculated when flour from the Polo cultivar was added (63.8%; 72.5%), likewise an increase in the EAAI calculated on the base of the FAO/WHO 1991 standard (104.7%). The highest EAAI was observed for the bread prepared with flour from the Markiz cultivar, when calculations were based on the DRI/USA 2005 standard (128.8%). The true digestibility of protein was slightly lower in all lupin enriched samples (86.2%) than in the control (86.6%). The PDCAAS increased considerably in the samples under analysis compared to the values calculated for the bread without the addition of lupin flour (37.3%; 42.4%). The largest increase was observed when flour from the Polo cultivar was added (55.0%; 62.5%). Similar values of the PER were calculated for the bread with the addition of flour from the Juno (2.73), Legat (2.75) and Markiz (2.77) cultivars, whereas the value for the bread with the addition of flour from the Polo cultivar was the same as for the bread from wheat-rye flour – 2.68 (Table 3).

Starvation, malnutrition and insufficient supply of nutrients are the problems that the contemporary world struggles with. There is a significant demand for food products of high nutritional value, obtained from stable and sustainable sources (Raikos *et al.*, 2014).

Staple foods in the majority of countries are breads of different kinds and other products made from flour. In general, one can claim that bread is the basic food, although its forms can be different in different cultures. Bakery products are made mainly from wheat flour. This material is highly valued because of its versatile physical and chemical properties, and the possibility of use in manufacturing many food products.

Table 3
Chemical and dietary indices of the protein nutritional value of wheat-rye bread made with selected lupin flours

Specification	Type of bread				
	Control K	**Juno J**	**Polo P**	**Legat L**	**Markiz M**
EP[1], %	3.9	5.6	6.7	5.4	5.7
EP[2], %	4.4	6.3	7.6	6.1	6.5
CS[1], %	43.1	55.2	63.8	50.0	51.7
Limiting amino acid[1]	Lys	Lys	Lys	Lys	Lys
CS[2], %	49.0	62.7	72.5	56.7	58.8
Limiting amino acid[2]	Lys	Lys	Lys	Lys	Lys
EAAI[1], %	100.0	102.3	104.7	102.3	102.3
EAAI[2], %	123.0	120.2	125.9	123.0	128.8
TD, %	86.6	86.2	86.2	86.2	86.2
PDCAAS[1], %	37.3	47.6	55.0	43.1	44.6
PDCAAS[2], %	42.4	54.0	62.5	48.9	50.7
PER	2.68	2.73	2.68	2.75	2.77

[1] Chemical indices of nutritional value of protein calculated on the basis of the amino acid standard FAO/WHO 1991.
[2] Chemical indices of nutritional value of protein calculated on the basis of the amino acid standard DRI/USA 2005
EP – effective protein; CS – chemical score; EAAI – integrated essential amino acid index; TD – true digestibility; PDCAAS – protein digestibility corrected amino acid score; PER – protein efficiency ratio

Discussion

A tendency has been recently observed to enrich cereal products, mainly those made from wheat and wheat-rye mixes, with flours obtained from other plants, such as lupin, green peas, beans, hemp and buckwheat. These flours are highly valued because of their functional properties, which include solubility, emulsifying capabilities, foaming and gelling properties, and water retaining capability (Raikos *et al.*, 2014). Economic, environmental and health-related factors play an important role in seeking new solutions and combinations in bakery. They include prices increase on the food market, the need to ensure supplies from stable and so far unused sources and preventing protein undernourishment. The last factor is a common problem in developing and economically backward countries due to restricted access to animal protein (Bhat & Karim, 2009). With this issue in mind, the present study focuses on the usability of lupin in a form of flour in bread baking as an opportunity to improve the nutritional value of protein contained in this food product. Since bread, in its various forms, is highly valued around the world owing to its sensory properties, readiness to be consumed and, importantly, because of its price, one can claim that it is eaten by all social groups (Correia *et al.*, 2015). Therefore, improving the quality of protein consumed with this product can contribute to the considerable improvement of nourishment of many people.

The present study has demonstrated an alteration of the quality of protein in the wheat-rye bread prepared with an addition of flour from different lupin cultivars. An addition of lupin flour itself contributed to an increase in the total protein content in the product (Table 1). Lupin seeds have been consumed since antiquity but, apart from being used as food, they have many other applications (EFSA 2005). Interestingly, a 5% addition of lupin flour from sweet lupin cultivars has a positive effect on the organoleptic properties of bread and it delays the process of bread staling (Skibniewska *et al.* 2003). An addition of 10% lupin flour to bread generates a product with slightly different physical and chemical properties. These include rheological properties of dough, its density, colour and texture. It is encouraging that the consumer sensory assessment showed that the differences originating from the enrichment with lupin flour are practically imperceptible, which makes it possible to improve the nutritional quality without changing the product features, which mainly affect the consumers' choices (Correia *et al.*, 2015). Lupin flour and protein isolates from lupin seeds are widely used in creating new food products because their addition does not affect the taste of a final product (Gresta *et al.*, 2017). Studies have shown that an addition of lupin flour to wheat bread improves considerably the quality and amount of protein and dietary fibre in a final product. The growing interest in the use of lupin seeds in the global food production is also caused by the fact that it is not a genetically modified plant and it contains less phytoestrogens than soybean, and also that lupin flour provides less calories than refined wheat flour (Hall & Johnson, 2004; Villarino *et al.*, 2015a). Lupin seeds are rich in carotenoids, phenols and have high antioxidant potential (Siger *et al*., 2012). In Arab countries, flour from seeds of leguminous plants is used to improve the nutritional value of pita bread (traditionally made from wheat flour), because of a low level of lysine and a relatively high level of sulphur amino acids in wheat protein (Mubarak, 2001; Abu–Ghoush *et al.*, 2008). Moreover, enriching Arab bread with lupin flour has contributed to an increase in the content of total ash, dietary fibre, total fat and a decrease in carbohydrates content (Al Omari *et al.*, 2015). Lysine was the limiting amino acid in the protein of the wheat-rye bread under study. The chemical score increased after the addition of lupin flour, by even 25% in the case of the Polo cultivar (Table 3). Lupin protein is described as complementary and it perfectly supplements the diet with essential amino acids (Lampart–Szczapa *et al.*, 2003; Erbas *et al.*, 2005). Their content is estimated to be higher than in soybean and regarded as a perfect substitute of animal protein (Süssmann *et al.*, 2007). However, although European consumers express positive opinions on the consumption of plant protein, they are largely unaware that lupin seeds are not inferior to soybean in this regard (Lucas *et al.*, 2015). Lupin is an alternative to soybean in the production of fermented foods, such as Indonesian tempeh, Japanese miso and natto, and fermented spices (Rybiński *et al*., 2018). Inclusion of lupin in the human diet also brings other benefits than just the quality of protein. Studies have confirmed the positive effect of the consumption of lupin flours in diabetes because of the presence of dietary fibre, and the properties which reduce the risk of coronary disease owing to the presence of agents which decrease the blood lipid and sugar levels (Duranti, 2006; Rumiyati *et al.*, 2012; Rumiyati *et al.*, 2015; Tadele, 2015). Currently, lupin seeds, flour and bran are used in Europe to enrich breads, pasta, cakes, muffins as well as extruded snacks and beverages. Lupin is also widely used in production of gluten-free food. Lupin germs are eaten in Australia. White lupin grown in Ethiopia under the local name of "Gibto" is baked and then used as raw material in production of alcoholic beverage called "Arekie". It is also used as a snack or a condiment called "Shiro" (Tizazu & Emire, 2010; Yeheis *et al.,* 2010). Efforts have also been made to replace animal and soy protein with lupin seeds in feeding pigs and broilers, which would have a beneficial effect on the fatty acid profile of pork and poultry (Zraly *et al.,* 2007; Mierlita 2015).

The dietetic role of lupin has been confirmed in studies on the possibility of effective regulation of appetite owing to an addition of lupin flour to wheat bread. Forty percent of wheat flour used to make dough was replaced with lupin flour. Analyses have revealed more than two-fold increase in the total protein content in bread with an addition of lupin compared to wheat bread, with an unchanged amount of total fat. Moreover, the amount of fibre increased almost four-fold. Bread with an addition of lupin flour was a rich source of protein and fibre, which partly replaced carbohydrates from wheat flour. The amount of carbohydrates was found to decrease by 30%. Consumption of bread enriched with lupin flour resulted in an increased feeling of satiety and lower (by approx. 20%) intake of energy in next meals. It affected secretion of ghrelin, glucose and insulin after a meal. This experiment has confirmed that the consumption of high protein diet rich in fibre reduces energy intake in subsequent meals more than consumption of high-carbohydrate diets (Lee *et al.,* 2006). Food enriching with lupin can be useful in treatment and prevention of obesity as well as some diseases caused by this state. As has been noted earlier in the present study, an addition of lupin flour to wheat-rye bread resulted in a considerable increase in the amount of arginine in the protein, regardless of which cultivar of lupin was used. A high content of arginine is typical of all lupin cultivars. This amino acid plays a key role in regulating lipid content in blood. A study conducted by Bähr *et al.* (2015) showed that consumption of products enriched with lupin protein (bread, sausages, vegetarian pâtés) has a beneficial effect on the blood lipid profile, in the same way as the consumption of products enriched with milk protein with an elevated concentration of arginine. Food enriched with lupin is said to have a nutraceutical potential, so it has a positive effect on human health in the treatment and prevention of diseases. Apart from these benefits, lupin proteins are known to decrease the sugar level in blood. It has been demonstrated that lupin protein extract is 10 times more potent than standard anti-diabetic drugs (Agrawal *et al.,* 2015). The main interest is focused on gamma-conglutin, which accounts for approx. 5% of all lupin seed proteins and which plays a role in the plants' defence against pathogens (Agizzio *et al.,* 2003). This bioactive peptide has not been found in bread made exclusively from refined wheat flour. In addition, such benefits like the facilitation of defecation and blood pressure decrease have been mentioned. Arginine is a precursor of the synthesis of nitrogen oxide. The mechanism of blood pressure decrease may involve relaxation of the blood vessel walls by nitrogen oxide, which is a potent endothelium relaxing factor (Sedlakova *et al.,* 2016). Other examples of bioactive lupin proteins include serine protease of the Bowman-Birk type, whose beneficial effect has been demonstrated in cancers, atrophy of skeletal muscles, angiogenesis, rheumatoid arthritis, neurodegenerative diseases and coronary disease treatments (Scarafoni *et al.,* 2008).

In the present study, the addition of lupin flour to wheat-rye bread considerably improved its *in vitro* digestibility (Table 1) and increased the PDCAAS (Table 3). A positive effect of the addition of flour from leguminous plants to wheat sourdough breads and gluten-free cakes on *in vitro* digestibility of proteins in the final product has been observed (Anyango *et al.,* 2011; Gularte *et al.,* 2012). Studies have shown that 5% addition of lupin flour increases the PDCAAS by 15 to approx. 50%, depending on the lupin cultivar used. The findings of Villarino *et al.* (2015b) indicate that a 20% addition of lupin flour from various cultivars of narrow leaf lupin (*Lupinus angustifolius*, ASL) increases the PDCAAS by approx. 50%, regardless of the cultivar used. Such high values of these indices make it possible to compare lupin proteins and proteins of leguminous plants to animal proteins (Erbersdobler *et al*., 2017a,b).

The effect of enriching bread with lupin flour on the protein efficiency ratio proved insignificant for all the lupin cultivars under study. The findings of animal studies presented by Monteiro *et al.* (2014) were similar and were indicative of a much greater effect of supplementation with casein on the PER.

Conclusions

The addition of lupin flour to wheat-rye bread resulted in an increase in total and digestible protein in the final product, regardless of the lupin cultivar used. Enrichment of wheat-rye flour with lupin flour from the Juno cultivar significantly increased the *in vitro* digestibility of protein in the bread. The greatest increase in the effective protein (EP), chemical score (CS), protein digestibility–corrected amino acid score (PDCAAS) and essential amino acid index (EAAI) was achieved when the bread was enriched with flour from Polo cultivar. Wheat-rye flour enriching with lupin flour from the Juno, Polo, Legat and Markiz cultivars, intended for bread making, did not have a significant effect on the protein efficiency ratio (PER). The addition of lupin flour in the process of bread making resulted in enrichment of the final products in essential amino acids lysine and leucine, and non-essential amino acids asparagine and arginine. Protein of low-alkaloid cultivars of lupin Juno, Polo, Legat and Markiz can be a valuable component which increases the nutritional value of bread protein and for this reason it should be recommended for the use in baking.

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