# The influence of lupine flour on selected parameters of novel bakery products

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**Abstract:** The aim of the present study was to evaluate the effect of lupine flour on selected parameters of novel bakery products. Different proportions of lupine flour – from 25% to 100% – were added to the baked biscuits; wheat flour biscuits were prepared as the control sample. Increasing lupine content has positively affected mineral concentrations and sensory properties. After 4 days of storage, the lupine biscuit samples showed no significant differences in their water activity values when compared with each other. Compared with the control sample, the concentration of Ca was considerably increased by 1.2% in the biscuits with 25% lupine flour addition, which represents 5.8% of the recommended daily intake of Ca contained in 100 g of biscuits. The biscuits containing 25% of lupine flour showed no significant difference in colour compared to the control sample. The intensity of the sensory properties of the biscuits increased by adding more lupine flour; all the samples of lupine biscuits were acceptable to the evaluators. Based on the results, 25% of lupine flour addition may be considered the most appropriate.

Keywords: biscuit; legume; minerals; sensory evaluation

Currently, there is a growing interest in the development of new value-added foods containing bioactive compounds. Improving nutritional properties of foods can be achieved by adding various ingredients; especially demanded are those from natural sources with minimum refining process (Wandersleben et al. 2018). Legumes are classified as an important group of crop plants, the seeds of which are nutritionally beneficial. Legume seeds are a rich source of proteins, minerals, vitamins and sugars; therefore, their biological value is high and widely used in human diet (Bojňanská et al.

2012). The annual consumption of legumes is about 1.2–2 kg per capita in Slovak Republic (Slovakia), while in other European countries it is about 2.2–3.4 kg per capita (Timoracká et al. 2010).

Lupine is a legume belonging to the family Leguminosae that consists of approximately 200–600 species; only four of which are used in agriculture for food and feed production (Prusinski 2017). Seeds of different varieties and species of lupines can vary greatly in size, colour (grey, white, brown) and shape (oval, flat, round) (Villarino et al. 2015). The most important

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species of lupines for human consumption are Lupinus angustifolius (blue lupine or Australian sweet lupine), Lupinus luteus (yellow lupine), Lupinus albus (white lupine) and Lupinus mutabilis (South American lupine) (Koeberl et al. 2018; Carvajal-Larenas 2019). Lupine contains many proteins with a balanced amino acid profile, which increases the interest in these seeds. Furthermore, it is a significant source of dietary fibre and negligible starch that are an indispensable part of a healthy diet (Wandersleben et al. 2018; Kohajdová et al. 2011). Starch content of lupine flour is much lower compared to wheat flour. Lupine grains are also a source of vitamins (thiamine, niacin, riboflavin, tocopherols) and antioxidants (polyphenolic tannins, flavonoids), proteins - referred to as globulins: α-conglutin (11S globulin), β-conglutin (7S globulin),  $\gamma$ -conglutin (7S basic globulin) and  $\delta$ -conglutin (2S albumin) - and amino acids (cysteine, methionine, valine, tryptophan, lysine, isoleucine, leucine, phenylalanine, and tyrosine). The protein content of lupine is significantly higher compared to other legumes (Bahr et al. 2014). Kaczmarek et al. (2014) indicated that lupine seeds, among other amino groups, contain a high portion of arginine. On the contrary, compared to wheat grains, lupine grains contain a lower amount of antinutrients such as phytates and saponins (Villarino et al. 2015).

The mineral composition of lupine is similar to that of other legumes and the content of mineral elements in lupine varies depending on the variety. Compared to other legumes, calcium (Ca) content in lupine was found to be present only in lower concentrations. On the other hand, manganese (Mn), iron (Fe) and zinc (Zn) are more abundant in lupine than in other legumes. For example, high concentrations of Mn were detected in the species L. albus (Trugo et al. 2003). Biscuits are potential food products suitable for functional addition of ingredients (Ivanišová et al. 2018). In their study, Jayasena et al. (2009) reported that adding 20% of lupine flour increases the protein content from 54% to 85% and the fibre content from 160% to 210% in biscuit samples. Therefore, 100 g of such biscuits (20% lupine content) provides the recommended daily intake of fibre: 70%, protein: 50% and of micronutrients: 30% (K, Mg, P, Fe).

The main objectives of this study were to evaluate the effect of lupine flour from Slovakia on biscuit production and quality of selected sensory parameters.

We hypothesised that increasing additions of lupine flour will positively affect the sensory properties and mineral content of bakery products.

### MATERIAL AND METHODS

#### Sample preparation

The main aim of the study was to analyse the biscuits with different contents of lupine flour and to compare them with the control sample that was prepared from 50 g of wheat flour T512, 34 g of vegetable shortening (Hera) and 17 g of powdered sugar. The biscuit samples with lupine flour from lupine grown in Slovakia were prepared with four different proportions of lupine flour (25, 50, 75, and 100% w/w) that was incorporated into the biscuit flour mixture by replacing the equivalent amount of wheat flour of the original recipe (the control sample). The dough (101 g) was rolled out to 0.5 cm thickness and cut into circular shapes of 5 cm in diameter (approximately 7 g). The cut biscuits were placed onto a baking tray and baked in an oven at 160 °C for 10 min. Biscuits were stored at room temperature (20 °C) for 13 days in closed plastic bags (polyethylene) suitable for food storing purposes, without presence of any inert gases. The biscuit samples were prepared in triplicate.

#### **Determination of general parameters**

The moisture content of the samples was determined with a moisture analyser (Kern DBS 60-3; Kern, Germany) according to the operational manual; for each analysis, 1 g of crushed sample was weighed. The water activity of the samples was determined with an apparatus for water activity measurement (LabMaster-aw; Novasina AG, Sweden) according to the operational manual; for each analysis, 0.5 g of crushed sample was weighed. Each sample was measured in duplicate and the results represent the average of six repeated measurements (maximum standard deviation  $\pm$  5%).

# Determination of mineral elements by ICP-OES

The samples (up to 0.5 g) were mineralised in the high performance microwave digestion system Ethos UP (Milestone Srl, Italy) in a solution of 5 mL HNO $_3$  for trace metal analysis (VWR Chemicals, Belgium), and 1 mL of  $H_2O_2 \geq 30\%$  for trace element analysis (Merck Suprapur®, Germany). The mineralisation mixture was filtered through the Sartorius (grade 390) filtration discs (Sartorius AG, Germany). After the sample pre-treatment process, the Árvay et al. (2019) method was applied for the quantification of elements (Ca, Fe, K, Mg, Mn, and Zn) using an inductively coupled plasma emission spectrophotometer (ICP-OES 720; Agilent Technologies, Australia). Each sample

was measured in duplicate, and the results represent the average of six repeated measurements (maximum standard deviation  $\pm$  5%).

#### Sensory evaluation

The sensory evaluation was carried out at the sensory laboratory of the AgroBioTech Research Centre SUA in Nitra; its infrastructure meets the requirements of international standard ISO 8589:2007. The evaluation was performed by 12 sensory assessors. For the sensory attributes of the product a 5-point intensity scale was applied. The 5-point hedonic scale was used for the overall acceptability assessment. The samples were served in the RCBD (randomised complete block design) order. The data were adjusted and results were processed using the nonparametric Friedman test and PCA (principal component analysis) acceptability assessment by individual assessors in the statistical package R (2000, R Foundation for Statistical Computing, Austria). The results were visualised using a boxplot graph. A description of the monitored sensory attributes follows below.

Round shape stability after baking. The ability of the product to retain its original round shape after baking (1 – decomposition of the original shape, 5 – preserved original shape).

Colour intensity. Colour change after baking (1 – light; 5 – very dark).

Odour intensity. The total intensity of the product odour (1 – weak, insignificant; 5 – strong, rich, intense). Odour intensity of legumes. Lupine-specific taste (1 – none; 5 – strong, intense).

Flavour intensity. The total intensity of the product flavour (1 – weak, indistinct; 5 – strong, rich, intense). Flavour intensity of legumes. Lupine-specific flavour (1 – none; 5 – strong, intense).

*Acceptability.* The overall positive impression of the product; the hedonic scale was used (1 – dislikes; 5 – very tasty, likes).

#### Statistical analysis

The Statgraphics Centurion (©StatPoint Technologies, Inc., USA) and GraphPad Prism 6.01 software (GraphPad Software Incorporated, USA) was used for the statistical analysis. The ANOVA (analysis of variance) method complemented by Tukey's Multiple Comparison Test with values of P < 0.05; P < 0.01; P < 0.001 was applied.

#### RESULTS AND DISCUSSION

The objective of this research part was to enrich durable biscuits with different amounts of lupine flour to increase their nutritional properties and to monitor such biscuits for their technological and sensory properties as well. The results were to lead to the optimisation of production. Wheat flour was replaced by lupine flour in the amounts of 25, 50, 75, and 100%.

The value of the water activity in the analysed fresh samples was measured in the range from 0.33 (the biscuits with 50% lupine content) to 0.40 (the biscuit control sample) (Table 1). There was no significant difference between 25% and 50% addition of lupine flour (P > 0.05). The biscuits were stored in closed plastic bags for 13 days. Considerable differences in water activity values were observed in lupine biscuits (25–100%) stored for 4 days, compared to the fresh samples under the same conditions (increase by 1.78 times). The highest values of water activity in the range of 0.70–0.71 and the highest moisture content of 8.12–10.3% were measured in the samples stored for 7 days. The water activity value (0.65–0.66) and moisture (7.06–8.97%)

Table 1. Average values of water activity and moisture of the biscuit samples (n = 6, SD  $\pm$  5%)

Sample	Fresh samples		Storage						
			4 days		7 days		13 days		
	water activity	moisture (%)	water activity	moisture (%)	water activity	moisture (%)	water activity	moisture (%)	
Control	0.40 <sup>a</sup>	2.94ª	0.63ª	6.97ª	0.70 <sup>a</sup>	8.12ª	0.66ª	7.06 <sup>a</sup>	
25% lupine	$0.34^{\rm b}$	$2.67^{b}$	$0.64^{\rm b}$	$7.59^{b}$	0.70 <sup>a,c</sup>	$9.05^{b}$	$0.66^{b}$	$7.68^{b}$	
50% lupine	$0.33^{b}$	$2.64^{b}$	$0.64^{\rm b}$	7.99 <sup>c</sup>	0.70 <sup>a,c,d</sup>	$9.37^{c}$	$0.65^{b}$	$8.34^{\rm c}$	
75% lupine	$0.34^{\rm c}$	1.98 <sup>c</sup>	$0.64^{b}$	$8.45^{d}$	$0.71^{b,c,e,f}$	$9.92^{d}$	$0.65^{b}$	$8.06^{d}$	
100% lupine	$0.38^{d}$	$3.09^{d}$	$0.64^{b}$	8.76 <sup>e</sup>	$0.70^{a,c,d,f}$	10.3 <sup>e</sup>	$0.65^{b}$	8.97 <sup>e</sup>	

Lupine – lupine flour; data with different superscript letters are significantly different (Tukey's multiple comparison test, P < 0.05; P < 0.01 or P < 0.001)

slightly decreased in the samples stored for 13 days compared with the results of the samples stored for 7 days. As each biscuit set (according to the different lupine contents) was stored only in one closed plastic bag for food storage purposes and without presence of any inert gases, the measurements showed unexpectedly fluctuating results. The increase of water activity during storage was also recorded in the research of Romani et al. (2016), where the biscuits were stored for 40 days. Due to the low water activity values, the biscuits have a long shelf life compared to other food products. However, the moisture uptake, high fat content, as well as aroma loss cause deterioration in the quality of the biscuits; therefore their good storage is necessary (Romani et al. 2016).

The visualisation of the shape stability proves a dominant position of the control sample and the sample with 25% of lupine flour. Applying the Friedman test, a statistically significant difference (P < 0.001) was detected between the groups of samples. Samples with higher

lupine content generated a higher variance in the attribute perception by the evaluators. When evaluating the visual attributes, there was a statistical difference in the perception of the intensity of the samples using the Friedman test (P < 0.001). Both visual attributes are shown in Figure 1. The difference in colour intensity is demonstrated in Figure 2. Jayasena et al. (2009) stated that the colour of biscuits is not significantly affected by additions of lupine flour up to 20% of the content. The evaluation of odour attributes showed the following results: when evaluating the intensity of the odour, the control sample was perceived as not very intense. The intensity of the overall odour increases with the increasing proportion of lupine flour. Identical results can be observed also by the fact that the samples show a statistically significant difference in the perception of the intensity of odorous substances by the Friedman test (P < 0.001). The results are shown in Figure 3.

The flavour attributes copy the results of the aromatic attributes in a very similar way. There is a statistical

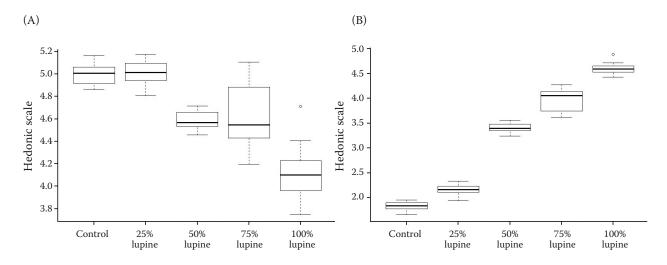


Figure 1. The visual attributes of the baked products: Round shape visual after baking (A) and intensity of the colour (B) Lupine – lupine flour

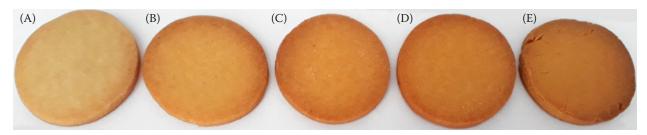


Figure 2. The overall appearance and shape of the biscuit samples: 0% lupine flour (A), 25% lupine flour (B), 50% lupine flour (C), 75% lupine flour (D), 100% lupine flour (E)

similarity between the control sample and the sample with 25% lupine flour content. Other samples are statistically significantly different (P < 0.001). The visualisation of the results can be seen in Figure 4. The overall acceptability of the product was assessed using a hedonic scale. Different acceptance intervals are used for the development of a new product. For sensory shelf-life acceptance tests the 9-point scale is commonly used, where the value of 6.5 is still an acceptable quality; this limit represents about 70% of the whole scale. As a new product with marginal raw materials is considered here, we set the limit for acceptability to 80% of the overall scale (black horizontal line in the graph).

The best rated product was a sample with 25% of the lupine flour. It reached the score 4.6 out of 5, when being evaluated by the panel consisting of 12 evaluators. The second best was the control sample (4.2 out of 5), and there was no statistically significant difference between them using the Friedman nonparametric test. The sample with 50% of the lupine flour content was also acceptable, but there was already a statistically significant difference (P < 0.05). Acceptability of the first three samples is highlighted by grey colour in Figure 5. Other samples were not accepted by the panel and were evaluated as statistically significantly worse. The result is demonstrated in Figure 5.

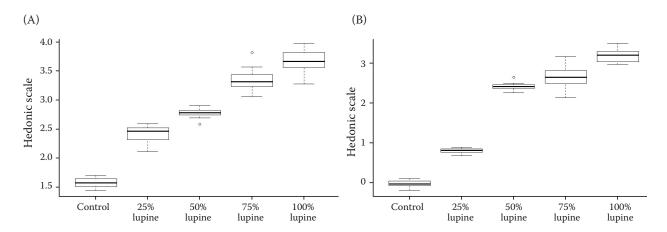


Figure 3. The aroma attributes of the baked products: Intensity of the aroma (A) and intensity of the legume aroma (B) Lupine – lupine flour

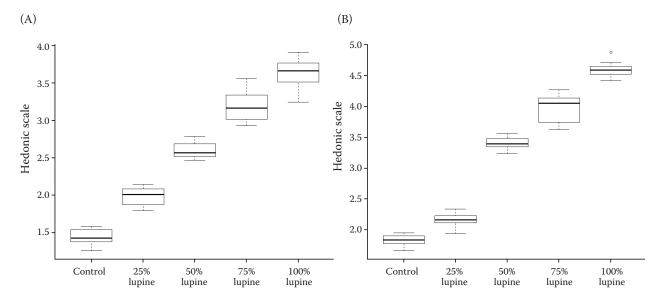


Figure 4. The flavour attributes of the baked products: Intensity of the flavour (A) and intensity of legume flavour (B) Lupine – lupine flour

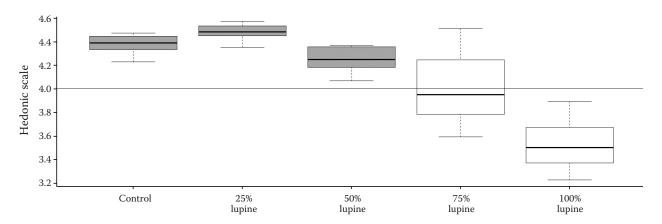


Figure 5. The overall acceptance of the baked products Lupine – lupine flour

For a comprehensive evaluation of the acceptability of the samples and their sensory attributes, the technique of data reduction (PCA) was selected. In Figure 6 it is possible to observe a strong relation between the shape and the overall acceptability of the product. On the contrary, very high values attributed to the colour, odour, and taste traits reduce the overall acceptability.

The composition of the mineral elements in biscuit samples and their statistical analyses are presented in Table 2. The biscuits made from wheat flour, without any addition of lupine flour, showed the lowest contents of minerals among all the samples analysed in the experiment. With increasing additions of lupine flour, the concentration of selected elements increased, respectively. Thus, the highest content of the elements was recorded in the biscuit sample made from 100% lupine flour. Based on the abovementioned results of the sensory evaluation, further attention was paid to the mineral content of the biscuits with 25 and 100% lupine flour content.

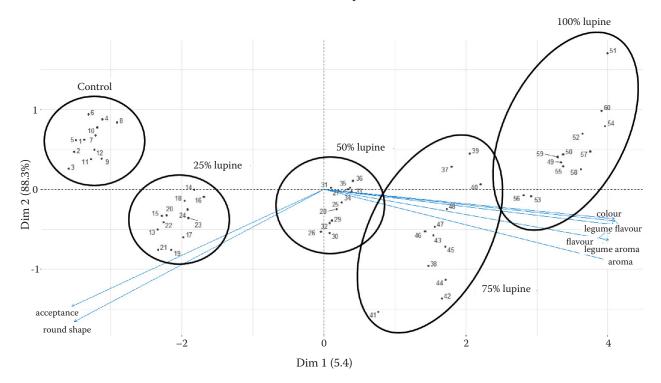


Figure 6. PCA acceptability assessment by individual sensors PCA – the principal component analysis; lupine – lupine flour

Table 2. Average concentrations of selected mineral elements in the biscuit samples (n = 6, SD  $\pm$  5%)

Sample	Elements (mg kg <sup>-1</sup> )									
	Ca	Fe	K	Mg	Mn	Zn				
Control	477 <sup>a</sup>	10.60 <sup>a</sup>	587.00 <sup>a</sup>	216 <sup>a</sup>	2.57 <sup>a</sup>	5.27 <sup>a</sup>				
25% lupine	581 <sup>b</sup>	$15.30^{b}$	$1.39^{b}$	371 <sup>b</sup>	$4.31^{b}$	$7.82^{b}$				
50% lupine	613°	$17.40^{\circ}$	$2.12^{c}$	502°	5.78°	9.98°				
75% lupine	$584^{b}$	$19.30^{\rm d}$	$3.33^{d}$	738 <sup>d</sup>	$7.86^{d}$	$14.40^{ m d}$				
100% lupine	696 <sup>d</sup>	22.50 <sup>e</sup>	$3.80^{e}$	798 <sup>e</sup>	9.15 <sup>e</sup>	17.00 <sup>e</sup>				

Lupine – lupine flour; Ca – calcium; Fe – iron; K – potassium; Mg – magnesium; Mn – manganese; Zn – zinc; data with different superscript letters are significantly different (Tukey's multiple compar–ison test, P < 0.05; P < 0.01 or P < 0.001)

The concentration of Ca was considerably increased by 1.2 and 1.5% in the biscuits with 25 and 100% content of lupine flour, respectively (P < 0.001), compared to the control sample (477 mg kg<sup>-1</sup> of Ca amount). The daily intake of Ca for adult men and women (19–50 years) is 1 000 mg (IOM 2011). The maximum daily Ca intake for men and women (19–50 years), as well as for pregnant and breastfeeding women, is 2 500 mg (Bailey et al. 2010). For example, 100 g of the biscuits with 25 and 100% addition of lupine flour could provide 5.8 and 7.0% of the recommended daily intake of Ca, respectively.

The concentration of Fe was significantly increased by 1.4 and 2.1% in the biscuits with 25 and 100% lupine flour content, respectively (P < 0.001), compared to the control sample (10.6 mg kg<sup>-1</sup> of Fe amount), as well as comparing the samples with each other. The recommended daily intake of Fe for adult men and women (19–50 years) is 8 mg day<sup>-1</sup>. The upper limit of the daily intake is 45 mg Fe day<sup>-1</sup> (IOM 2011). In this case, 100 g of the biscuits with 25 and 100% addition of lupine could provide 3.4 and 5.0% of the recommended daily intake of Fe.

The concentration of K was significantly increased by 2.4 and 6.5% in the biscuits with 25 and 100% lupine flour content, respectively (P < 0.001), compared to the control sample with 587 mg kg $^{-1}$  of K, also comparing the samples with each other. The recommended daily intake of K for adult men and women (19–50 years) is 4.7 g (IOM 2011). WHO (2012) stated that the maximum daily allowance for K was not defined. 100 g of the biscuits (25 and 100% addition of lupine) could provide 3.0 and 8.1% of the recommended daily intake of K.

The concentration of Mg was considerably increased by 1.7 and 3.7% in the 25 and 100% lupine flour enriched biscuits, respectively (P < 0.001), compared to the control sample (216 mg kg<sup>-1</sup> of Mg content), also comparing the samples with each other. For adult men and women (19–50 age), their recommended daily

intake is 400 mg (IOM 2011). The maximum daily intake of Mg for men and women (over 19 years), as well as for pregnant and breastfeeding women, is 350 mg (IOM 1997). 100 g of the biscuits (25 and 100% addition of lupine) could provide 9.3 and 19.9% of the recommended daily intake of Mg.

The concentration of Mn was statistically increased by 1.7 and 3.6% in the biscuits with 25 and 100% lupine flour content, respectively (P < 0.001), compared to the control sample (2.57 mg kg<sup>-1</sup>), also comparing the samples with each other. For adult males and females (19–50 years) an adequate intake of Mn was set to 2.3 mg day<sup>-1</sup>. The tolerated upper limit of the daily Mn intake was set to 11 mg day<sup>-1</sup> with no observed adverse effect on the human organism (Jayasena et al. 2008). In this case, 100 g of biscuits (25 and 100% addition of lupine) could provide 18.7 and 39.8% of the recommended daily intake of Mn.

The concentration of Zn was considerably increased by 1.5 and 3.2% in the biscuits with 25 and 100% lupine flour enhancement, respectively (P < 0.001), compared to the control sample (5.27 mg kg<sup>-1</sup>) and also comparing the samples with each other. For adult males and females the recommended daily dose of Zn is 11 mg day<sup>-1</sup>. The upper Zn dose per day is 40 mg (Jayasena et al. 2008). 100 g of the biscuits (25 and 100% addition of lupine) could provide 4.8 and 21.2% of the recommended daily intake of Zn.

## **CONCLUSION**

Currently, products with low wheat flour contents are preferred. This study shows a possibility to make biscuits with lower gluten amounts and gluten-free biscuits with lupine flour. The present study provides experimental evidence that the flour from lupine grown in Slovakia might be a potential source of health-promoting agents. The content of lupine flour has positively

affected mineral concentrations and sensory properties, so the stated hypothesis of the research was confirmed. Another subject of the study could be to monitor the influence of different varieties of lupine and how to improve the parameters using herbs and spices.

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