Development and evaluation of potential functional food biscuits made from White Lupin

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ABSTRACT

Potential functional food bakery products were developed and characterized based on White Lupin (*Lupinus albus* cv. Nelly) flour. Analytical properties of the seeds resemble to previously described *Lupinus* species, with significantly high protein content (45%). The high protein and dietetic fiber content of the seeds makes Lupin flour suitable to develop potential functional food products with high nutritional values. Results of the development of sweet biscuits and salty crackers enriched with Lupin flour are presented. Sensory evaluation of the bakery products was carried out by 15 panelists using the nine points hedonic scale. Heat stability of White Lupin proteins were investigated by gel-electrophoretic analysis, White Lupin proteins are quite stable at 140°C, after 35 min heating the biscuits still contain 69% of the original amount of proteins. Baking conditions were optimized also based on gel-electrophoretic experiments, the optimal baking time was 30 min at 140°C. Gluten-free Lupin-based biscuits and crackers were produced by completely omitting wheat flour from the recipes.

KEYWORDS

White Lupin, functional food, gluten-free, gel electrophoresis

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INTRODUCTION

Grain legume seeds can be used for human and animal consumption or for the production of oils for industrial uses. Beans, lentils, lupins, peas, and peanuts are grain legumes (Hall et al., 2017). Legume seeds provide valuable proteins, therefore they have importance in human, as well as in vegan nutrition (Krintiras et al., 2016). Legume proteins contain high amount of lysine but only low amount of sulfur-containing amino acids. However, cereal proteins contain low amount of lysine, but appropriate amounts of sulfur-containing amino acids (Patil et al., 2016; Eggum and Beame, 1983). Using legume proteins in bakery products is therefore a possible way to improve the amino acid composition of the products. Soybean protein preparations (Bashir et al., 2015; Ribotta et al., 2005), chickpea flour (Gómez et al., 2008), germinated chickpea flour (da Costa et al., 2020; Fernandez and Berry, 1989), germinated pea flour (Bashir et al., 2015; Sadowska et al., 2003) as well as Lupin flour (Villarino et al., 2016; Pollard et al., 2002; Doxastakis et al., 2002; Dervas et al., 1999) were applied for the preparation of different bakery products. Lupin is a protein containing foods, like peanut and soybean, that may trigger an allergic reaction in a small percentage of the population. Some people who are allergic to peanuts may also react to lupin (Villa et al., 2020).

The genus *Lupinus* comprises above 200 species, but only a few species have become domesticated and cultivated on a large scale. Lupin is an economically and agriculturally valuable plant which can be grown in different soils even at cooler climates compared with soy. Lupin has a strong capability for fixation and organic phosphorus release from soil and can be used in crop rotation during intensive grain production (Duranti et al., 2008; Fan et al., 2002; Honeycutt, 1998).

Lupin is a grain legume with high protein content (more than 35 m m⁻¹%) and low oil content, similarly to soybean (Lucas et al., 2015; Sujak et al., 2006). Its main advantage compared with other legumes is the low amount of anti-nutritional components (alkaloids, phytate) (Getachew et al., 2012) and lupin seeds lack other common anti-nutritional factors such as lectins and protease inhibitors (Lilley, 1999). Lupin is a good source of nutrients, not only proteins but lipids, dietary fiber, minerals and vitamins (Van de Noort, 2017; Martinez-Villaluenga et al., 2006; Torres et al., 2005; Petterson, 1998). Furthermore, Lupin contains phytochemicals with antioxidant capacity, such as polyphenols, mainly tannins and flavonoids (Król et al., 2018; Oomah et al., 2006). This legume has considerable cholesterol and glucose-lowering capacities (Ward et al., 2020; Duranti et al., 2008; Doxastakis et al., 2007; Makri et al., 2005; Mavrikakis et al., 2003). Its proteins are mainly globulins that represent about 90% of the total protein content (Czubinski and Feder, 2019; Nadal et al., 2011). The utilization of Lupin can be extended to the production of protein concentrates, that can be promising food additives with their enriched nutritional value for the development of potential functional foods (Arnoldi et al., 2015; Archer et al., 2004; Dijkstra et al., 2003; Linnemann and Dijkstra, 2002; Marrs, 1996; Batterham et al., 1986). Effort to obtain Lupin protein concentrates containing 60–70% crude protein has been made by many research groups (Vogelsang-O'Dwyer et al., 2020; Chapleau and Lamballerie-Anton, 2003; Mubarak, 2001; Wasche et al., 2001). Protein concentrates can be used as a substance for enriching different kinds of products, such as pastries, breads, and milk.

Lupin proteins are gluten-free, therefore this legume can be suitable for the development of gluten-free food products, that can be consumed by people having wheat allergies, coeliac disease (CD) or other gluten related disorder (Taraghihah et al., 2020). Food products may

bear the term gluten-free if the gluten content does not exceed 20 mg kg^{-1} , between 20 and 100 mg kg^{-1} gluten-content they should bear the term “very low gluten” according to the EC Regulation (Commission Regulation (EC), 2009). Gluten is the scientific name for the CD-activating proteins in wheat, it is composed of 2 main protein fractions: gliadins and glutenins (Capriles and Areas, 2014). In this paper we report on the development of wheat-based and gluten free biscuits and crackers based on white Lupin flour available in Europe.

MATERIALS AND METHODS

Raw materials

White Lupin (*Lupinus albus* cv. Nelly) was purchased from The Center for Agricultural and Applied Economic Sciences of University of Debrecen. Seeds were ground fine in a domestic mill (Widu 1, Germany), and the flour was filtered with 0.4 mm filter. White Lupin flour was added in different ratio to wheat flour. Corn starch was ordered from National Starch (BAKA SNAK E type) and gluten was provided by DETK biscuit manufacturer (Hungary). All other components (wheat flour (BL-55), sugar, salt, tartaric acid, etc.) are commercially available ingredients (Table 1).

Chemical analysis

Nitrogen content was determined by using the Kjeldahl method and was multiplied by a factor of 5.7 to determine protein content in lupin seeds (Hudson et al., 1976). Moisture content was determined by heating samples at 105°C to constant weight. Fat, moisture, protein and vitamin B content was expressed on a dry weight basis.

Fat and fatty acid analysis

Full fat white Lupin flour (5 g) was suspended in 30 mL hexane. The suspension was sonicated for 25 min at room temperature, then centrifuged at 4,000 g for 20 min. The extraction was repeated 2 more times with 30–30 mL hexane. The hexane solutions were evaporated to dryness by rotary evaporator. The aliquot (1 mL) was dissolved in isooctane, and ester derivative was prepared with 2M KOH in methanol and neutralized with NaHSO_4 . The analysis of fatty acid methyl-esters were performed by GC-MS (Shimadzu, 2010; Supleco-wax column). FAME-mix and Grain-mix standards were used (Sigma-Aldrich). The analyses were repeated 3 times with different white lupin seed samples, the results are the average of 3 runs (Table 2).

Table 1. Source of raw materials

Material	Source
White Lupin	The Center for Agricultural and Applied Economic Sciences of University of Debrecen
Corn starch	National Starch
gluten	DETK biscuit manufacturer (Hungary)
All other components (wheat flour (BL-55), sugar, salt, tartaric acid, etc.)	Confectionery supply specialty store (Eger, Hungary)

Table 2. Fatty acids in *Lupinus albus* cv. Nelly

Fatty acid*	Tr (min)	%
myristic acid	12	0.26
palmitic acid	16.3	16.79
palmitoleic acid	17.1	0.38
cis-10-heptadecenoic acid	20.7	2.06
stearic acid	24.2	42.54
oleic acid	25.3	1.8
linoleic acid	28	10.15
linolenic acid	32.7	8.43
arachidic acid	39.5	6.9
cis-11-eicosenoic acid	41.2	3.44
behenic acid	52.4	6.6
erucic acid	53.7	0.65

*Composition of Lupin seed oil: Saturated (73.09%), Unsaturated (26.91%), n-3 (8.43%), n-6 (10.15%), n-3/n-6 (0.83).

Determination of vitamins B₁, B₂, B₃, B₆, B₁₂ (Antakli et al., 2015)

Lupin flour (1 mL) was mixed with 4 mL n-hexane and 16 mL HPLC grade water. The mixture was vigorously stirred for 30 min and was centrifuged at 4,000g for 20 min. Water phase was separated and filtered with 0.45 μm membrane filter. The sample (20 μL) was measured by HPLC-UV at 254 nm. Column: C18 BDS (100 × 4.6 mm; 3 μm); mobile phase: solvent (A): 5.84 mM of hexane-1-sulfonic acid sodium:acetonitrile (95:5) with 0.1% triethylamine at pH 2.5 and solvent (B): 5.84 mM of hexane-1-sulfonic acid sodium:acetonitrile (50:50) with 0.1% triethylamine at pH 2.5; flow rate: 1.6 mL min⁻¹; column temperature: 40°C.

Preparation of white lupin total protein extract (Sironi et al., 2005)

Lupin protein isolate (LPI) was prepared by alkaline water extraction and isoelectric precipitation. Lupin flour (10 g) (*L. albus*) was suspended in 100 mL of distilled water then pH was adjusted to 9.0 using 1M NaOH. The suspension was stirred for 1 h at room temperature, then centrifuged at 8,000 g for 20 min. Extraction and centrifugation were repeated 2 more times. The extracts were combined and cooled to 4°C and acidified to pH 4.5 using 1 M HCl. The precipitate was recovered by centrifugation at 4°C at 8,000 g for 20 min, then neutralized with 1 M NaOH to pH 7.0 and washed with distilled water 3 times. Then the neutralized precipitate was freeze-dried.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

White Lupin protein isolates and biscuits were electrophoresed under reducing conditions, using a vertical gel electrophoresis apparatus (Cleaver Scientific, Omni Page mini). The Gels consisted of 5% stacking and 12% separating polyacrylamide gels and were electrophoresed at 10 mA current at stacking gel and 20 mA current at separating gel for 2.5–3 h. Gels were fixed with trichloroacetic acid (TCA) (12v/v) and then treated with PAGE washing solution (water:ethanol:acetic acid 75:10:5 v/v/v). The gels were stained with Coomassie blue PAGE staining

solutions (Fermentas) for 1 h and washed with water. Gel photos were made with a Biodoc apparatus (BioDoc-It-TM Imaging System) and were analyzed with Biodoc software (Doc-It-LS Image Analysis Software). Protein band volumes were determined by summarizing the grey values of the pixels and were corrected with the background.

Heat stability of white lupin proteins during baking and optimizing baking conditions

Baking parameters were optimized according to the data of heat stability of White Lupin proteins (Table 3). Lupin biscuits were made from full fat white lupin flour, corn starch and water. Batches were baked in an oven at 140°C for 20, 25, 30 and 35 min, at 150°C for 20, 25, 30 and 35 min, at 160°C for 20, 25, 30 and 35 min and at 170°C for 20, 25, 30 and 35 min. Sample biscuits from each batch were pulverized in domestic grinder, added (15 mg) to 1 mL sample buffer and were vortexed for 1 h. Aliquots of the extracts were analyzed by SDS – PAGE. Gels were loaded with white lupin total protein extract, raw biscuit dough extract, biscuits and protein ladder (Fermentas, PAGERuler, broad range, 5–250 kDa). The sample buffer consisted of 0.25 mol L⁻¹ Tris-HCl, pH 6.8, 7.5 mL/100 mL glycerol, 20 mg mL⁻¹ SDS and 65 mmol L⁻¹ 1,4-dithiothreitol (DTT). The intensity of protein bands was compared with the unbaked biscuit dough extract. To compare protein band intensities we selected distinguishable, well separated protein bands. The 44 and 47 kDa sized double protein band is suitable for this purpose. The protein bands of baked biscuits were compared with bands of raw biscuit dough sample. Protein band volumes were determined by summarizing the grey values of the pixels of the protein band and were corrected with the background. Protein band volumes were determined with Biodoc software.

Preparation and sensory evaluation of lupin biscuits

For comparison purposes, lupin free biscuits and crackers were produced (Prokisch et al., 2017). The ingredients for reference sweet biscuits: 100 g wheat flour, 0.07 g NH₄CO₃, 0.63 g NaHCO₃, 0.06 g salt, 30 g sugar, 0.52 g tartaric acid, 2.19 g gluten, 10 g butter, 37.5 mL water. The ingredients for reference salty crackers: 100 g wheat flour, 10 g rice flour, 0.06 g NH₄CO₃, 0.6 g NaHCO₃, 4 g salt, 0.4 g sugar, 0.5 g tartaric acid, 2.5 g gluten, 20 g butter, 40 mL water (DETK Biscuit Manufacturer recipes). Gluten-free biscuits were prepared without using wheat flour and gluten in our recipes. In the case of gluten-free sweet Lupin biscuits instead of 100 g wheat flour Lupin flour and starch were used in different ratios, altogether 100 g. In salty crackers 100 g Lupin flour and rice flour mixture was applied instead of wheat flour. Biscuits were evaluated by fifteen panelists with practice of sensory evaluation. Samples were rated on the nine points hedonic scale (Yu et al., 2007). Sensory scores: Like extremely = 9, Like very much = 8, Like moderately = 7, Like slightly = 6, Neither like nor dislike = 5, Dislike slightly = 4, Dislike moderately = 3, Dislike very much = 2, Dislike extremely = 1.

Table 3. Relative protein band intensities (SDS-PAGE) during biscuit baking

Temperature (°C)	20 min	25 min	30 min	35 min
140	99%	99%	99%	69%
150	98%	92%	38%	31%
160	85%	53%	33%	11%
170	82%	n.d.	n.d.	n.d.

RESULTS AND DISCUSSION

Composition of White Lupin seeds

White Lupin “Nelly” seeds contain 12% moisture, 45% crude protein, 9% oil and 18% fiber. White Lupin seeds did not contain vitamins B₁ and B₁₂, but vitamins B₂, B₃ and B₆ were detected in high amounts. In 100 g of White Lupin seed we found 3.46 mg of B₂, 4.34 mg of B₃ and 1.18 mg of B₆ vitamins.

Table 2 shows that the fatty acid content of Lupin seed consists of 73% saturated fatty acids and 27% unsaturated fatty acids. The high content of oil in Lupin seeds, together with a high proportion of unsaturated fatty acids (Table 2) are desirable for human nutrition. The high amount of n-3 and n-6 fatty acids and their favorable ratio is prominent among other legumes (Jahreis et al., 2016).

The composition of fatty acids in White Lupin seed oil was determined by gas chromatography. Stearic acid (42.54%) and palmitic acid (16.79%) are the predominant fatty acids in Lupin seed oil, but among other fatty acids the saturated myristic acid (12.0%) and the essential unsaturated linoleic (10.15%) and linolenic acid (8.43%) were in high percentage as well.

Heat stability of White Lupin proteins

White Lupin proteins are quite stable at 140°C, after 35 min heating the biscuits still contain 69% of the original amount of proteins. After 25 min baking time at 150°C the intensity of the protein band drops significantly, after 30 min of baking over 60% of the proteins decomposed. At 160°C, after 20 min baking time 15%, after 25 min nearly half of the initial protein amount was decomposed. After 25 min baking time at 170°C there were no detectable protein bands. Based on these experimental data, the optimal baking time is 30 min at 140°C or 25 min at 150°C. No new protein bands can be observed and all protein bands were fading equally. The relative protein band volumes are presented in Table 3.

Sweet lupin biscuits

The ratios of wheat flour, Lupin flour, gluten, starch and water were varied and the produced biscuits were evaluated in our experiments. The amount of wheat flour, Lupin flour and starch were always 100 g. The compositions of sweet biscuits with their sensory scores are shown in Table 4.

Biscuits were baked at different temperatures. Applying 20 min baking time at 130°C (Batch 1) biscuits scored low on sensory evaluation, because of strong bitter Lupin flavor and pale color. The increase of baking temperature to 140°C resulted in weaker Lupin flavor (Batch 2), at 150°C the Lupin flavor completely disappeared (Batch 3). At this temperature most of the proteins are still intact, there is no Lupin bitter flavor and biscuits had pale yellow color, because of Maillard reaction. Batches 3–13 were baked at 150°C for 25 min. Increasing the ratio of Lupin flour, biscuits became slightly harder (Batches 4, 5 and 6). Batches 10–13 are vanilla flavored biscuits, which were produced by using 10 g of vanilla flavored sugar and 20 g sugar instead of 30 g sugar. Attempts were made to produce gluten free Lupin biscuits (Batches

Table 4. Composition and sensory evaluation of sweet lupin biscuits

Batch No.	Wheat flour (g)	Lupin flour (g)	Corn starch (g)	Gluten (g)	Water (mL)	Note	Sensory score
Reference	100	-	-	2.19	37.5	130°C	7.8
1	80	20	-	2.19	37.5	130°C	5.2
2	80	20	-	2.19	37.5	140°C	5.9
3	80	20	-	2.19	37.5		7.1
4	60	40	-	2.19	37.5		7.3
5	40	60	-	2.19	35		6.3
6	20	80	-	2.19	35		5.1
7	-	50	50	-	35		5.5
8	-	70	30	-	30		5.3
9	-	60	40	-	35		5.6
10	50	40	10	2.19	35	vanilla flavored	7.9
11	-	50	50	-	35	vanilla flavored	6.4
12	-	70	30	-	35	vanilla flavored	6.1
13	-	60	40	-	35	vanilla flavored	7.1

Ingredients not indicated: 0.07 g NH_4CO_3 , 0.63 g NaHCO_3 , 0.06 g salt, 30 g sugar or 20 g sugar and 10 g vanilla sugar, 0.52 g tartaric acid, 10 g butter.

Note: Batches 7, 8, 9 and 11, 12, 13 are gluten-free.

7–9 and 11–13). The wheat flour was substituted with corn starch (National Starch, BAKA SNAK E type). The dough of biscuits made from Lupin flour and corn starch is thick and hard to handle, but after baking the products had acceptable texture and taste. Among Lupin enriched sweet biscuits Batch 4 and 10 scored the highest. The best score in the case of gluten-free Lupin biscuits was achieved in Batch 13, although it is slightly hard, its texture needs further development.

Salty lupin crackers

The ratios of wheat flour, Lupin flour, rice flour, gluten, corn starch and water were changed. The amount of wheat flour, Lupin flour, rice flour and corn starch were always 110 g. The compositions of crackers are shown in Table 5. All salty crackers were baked at 150°C for 20 min.

In the first nine crackers we omitted the rice flour completely. At low proportion of Lupin flour we experienced no changes in the texture and taste of crackers (Batches 1 and 2). At higher Lupin flour ratio, the crackers were slightly harder. By adding corn starch, rice flour or both to the dough, crackers became crunchier. Crackers with different wheat flour, Lupin flour, rice flour and corn starch ratios were produced. Best scoring products were Batch 10, 11 and 12. Among gluten-free crackers Batch 16 scored the highest on sensory evaluation. All these products were prepared from 40 g lupin flour.

Table 5. Composition and sensory evaluation of salty lupin crackers

Batch No.	Wheat flour (g)	Lupin flour (g)	Rice flour (g)	Corn starch (g)	Salt (g)	Gluten (g)	Water (mL)	Sensory score
Reference	100	-	10	-	4	2.5	40	7.8
1	100	10	-	-	4	2.5	40	7.2
2	90	20	-	-	3.5	2.5	40	7.1
3	70	40	-	-	3.5	2.5	40	6.6
4	55	55	-	-	4	2.5	40	5.5
5	-	55	-	55	4	-	40	5.5
6	-	70	-	40	4	-	40	5.2
7	70	30	-	10	4	2.5	40	7.0
8	55	55	-	-	3.5	2.5	40	6.3
9	60	40	-	10	3.5	2.5	40	6.9
10	50	40	10	10	3.5	2.5	40	7.7
11	50	40	-	20	4	2.5	40	7.6
12	55	40	-	15	4	2.5	40	7.6
13	45	35	-	30	4	2.5	43	6.5
14	60	40	10	-	4	2.5	42	7.3
15	-	40	30	40	4	-	40	6.5
16	-	40	30	40	4	-	45	7.1

Ingredients not indicated: 0.06 g NH_4CO_3 , 0.6 g NaHCO_3 , 0.4 g sugar, 0.5 g tartaric acid, 20 g butter.

Note: Batches 5, 6 and 15, 16 are gluten-free.

CONCLUSIONS

Full fat White Lupin flour is a good source of nutrients; it has high protein content and good amino acid composition. Lupin seeds do not contain gluten proteins that makes Lupin flour a feasible ingredient to make gluten-free products. In this paper we showed that full fat White Lupin flour is suitable to produce gluten-free sweet biscuits and salty crackers with good sensory evaluation values, acceptable texture and taste. Although these products are slightly harder compared to the corresponding wheat flour products, further developments can result in new Lupin-based gluten-free products.

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