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Technological and chemical characteristics of breads made with lupin sprouts

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RESEARCH ARTICLE

Abstract

In this study, the effect of sprouting processes on the physical and chemical properties of lupin flour and the usage of sprouted lupin flour (SLF) in bread making, and technological properties of breads supplemented with SLF were investigated. For SLF preparation, lupin seeds were sprouted at day 1, 3 and 6, and dried then finely ground. 10% lupin flours (sprouted and non-sprouted) replaced wheat flour in the bread formulation. After the sprouting process, flour properties (colour, ash, protein, phytic acid (PA) and mineral content) and bread properties made with these flours (weight, volume, specific volume, symmetry, texture, porosity, firmness, crust and crumb colour) were investigated. During the sprouting period, the PA content of SLF significantly ($P<0.05$) decreased. Potassium, manganese, phosphorus and zinc contents of the SLF increased throughout the sprouting period. Breads supplemented with 3 day SLF give superior technological (volume, specific volume, symmetry and texture) and nutritional properties compared to the control sample. The use of 3 day SLF can be recommended for nutritional enrichment of bread and also for improving technological quality with minimum adverse effect of bread colour.

Keywords: bread, sprouted lupin flour, phytic acid, mineral, technological properties

1. Introduction

Today, many new products are based on fortifying wheat flour (WF). To improve nutritional, technological and functional properties, some of the important components are protein, dietary fibre, mineral and flavonoids which can be used to fortify the WF in baked products. These products have a positive impact in terms of health and being natural. In the literature, it has been reported that functional properties of sprouted seeds and sprouts also increased with the addition of some of the above-mentioned components of these products (Arvanitoyannis *et al.*, 2005; Öztürk, 2008; Siro *et al.*, 2008; Sloan, 2000, 2002). Sprouting, the practice of germinating seeds to be eaten raw or cooked, is the beginning of growth of a plant from a previously dormant seed which contains the embryo. Several studies have reported that germination improves the nutritional properties of cereals and legumes (Hansen *et al.*, 1989; Marero *et al.*, 1989a,b). Benitez *et al.* (2013) reported that physicochemical properties of sprouted legume flours can improve oil holding, water holding, water absorption and gelation capacities, but decreases can occur in emulsifying and foaming capacities. During sprouting a decrease in the amount of anti-nutritional substances – phytic acid (PA), trypsin inhibitor, etc. – and an increase in the compounds with phytochemical properties have been reported by Sangronis and Machado (2007).

Sweet lupin seeds can be used as a protein and dietary fibre source to improve the nutritional value of end products (Hall and Johnson, 2004; Torres *et al.*, 2006), and also used for their emulsifying, binding and solubility properties (Sathe *et al.*, 1982; Sosulski *et al.*, 1978). Lupin flour is largely used as an egg replacer in pancakes, biscuits and brioche (Tronc, 1999), cookies and breads to improve the texture, flavour and colour (Dervas *et al.*, 1999; Gomez Galan *et al.*, 2011; Paraskevopoulou *et al.*, 2010, 2012) also added to spaghetti pasta (Capraro *et al.*, 2008; Doxastakis *et al.*, 2007; Martinez-Villaunenga *et al.*, 2010; Rayas-Duarte *et al.*, 1996) and crisps (Lampart-Szczapa *et al.*, 1997). Lupin flour is used in gluten-free foods (Levent and Bilgiçli, 2011; Scarafoni *et al.*, 2009; Ziobro *et al.*, 2013), because it does not contain gluten (Kohajdova *et al.*, 2011). El-Adawy *et al.* (2001) suggested another uses of lupin and that lupin protein isolates can be used in many food systems as a functional agents. Lupin is an alternative to soya bean and can be used as main food ingredient and milk replacer for vegetarians. Lupin fibre is also used to enrich the fibre content of baked goods and pasta (Clark and Johnson, 2002; Hall *et al.*, 2005; Smith *et al.*, 2006), besides addition of lupin flour could improve the water binding, texture, shelf life and aroma (Fudiyansyah *et al.*, 1995; Martinez-Villaluenga *et al.* 2006).

The addition of dietary fibre sources improves technological (rheological properties of flour, bread weight, loaf volume, specific volume, firmness, crust and crumb colour, crumb texture) and nutritional characteristics (vitamins, minerals high antioxidant content), maintenance of freshness during shelf-life, and increases dietary fibre content or manipulates process parameters such as mixing time of breads (Almeida *et al.*, 2013; Blandino *et al.*, 2013). The objective of this study was to investigate the effects of sprouted lupin flour (SLF) on bread quality and nutritional properties to increase bread properties made with SLF.

2. Materials and methods

Materials

WF, salt, yeast, SLF and water were incorporated into the formulation. The flour was a commercial WF (Hekimoğlu Flour Mill A.Ş., Konya, Turkey). Baker's yeast (Pakmaya Baker's Yeast Factory, Izmit, Turkey) and refined salt were purchased from a local market, and the lupin seeds were provided by a local pulses firm.

Methods

Sprouting process

This process was performed with some modifications of the method given by Frias *et al.* (2005). 200 g of lupin seeds were treated for 30 min with 1000 ml of 0.07% sodium hypochlorite. Then the seeds were washed with distilled water until the pH was neutral. Afterwards the seeds were soaked with 1000 ml of distilled water for 5 h and 30 min and shaken every 30 min. The hydrated seeds were placed on laboratory paper and they were then covered with the same wet paper. The seeds were sprouted in darkness at 20 °C for 1, 3, and 6 days. To keep the paper wet, the seeds were watered during the sprouting period. The sprouted seeds and the sprouts were dried with air condition oven (Nüve KD 400, Istanbul, Turkey) at 45 °C, ground and passed through a sieve of 0.5 mm. The flours obtained were stored in darkness, in deep freeze at -18 °C until further analysis.

Bread making procedure and tests

The bread ingredients used were 3.0% yeast, 2.0% salt, 10.0% SLF (all based on percentage of flour weight). All the ingredients were mixed using a Hobart mixer (Hobart N50; Hobart, Canada Inc., North York, Ontario, Canada). A straight dough process (AACC 10-10) was used with some modification (bulk fermentation: 30 + 30 min; proofing: 60 min) (AACC, 1990). After that the dough was baked at 235 °C for 15 min in a commercial oven (Arçelik ARMD-580; Arçelik, İstanbul, Turkey). The bread was cooled to room temperature and placed in polyethylene bags until tested. The external and internal bread properties (loaf weight and volume (rapeseed displacement method), specific volume, crumb texture, crumb and crust colour were determined (AACC, 1990). Specific volume (ml/g) was calculated by dividing loaf volume to loaf weight. The firmness of breads was measured in Newton's per square centimetre by a texture analyser using the procedure of Aydın and Öğüt (1991). The properties of symmetry, texture and porosity of breads were carried out under white light. Seven panellists (aged 25-35) assessed the sensory attributes of the bread samples. They were asked to evaluate each bread sample using a 5 point scale ranging from 1 (dislike) to 5 (like extremely). The samples were coded with numbers and served to the panellists at random to guard against any bias.

Laboratory analyses

The colour values of WF, non-sprouted lupin flour (NSLF) and SLF, crumb and crust colour of the bread samples were determined by measuring the L* (100 = white; 0 = black), a* (+ = red; - = green) and b* (+ = yellow; - = blue) values using a Hunter Lab Color QUEST II Minolta CR-400 (Minolta Camera, Co., Ltd., Osaka, Japan) with illuminant D65 as reference. The hue angle (h), which describes the hue or colour of a sample, was calculated (arctan(b*/a*)), as was the saturation index (chroma = $(a^{*2} + b^{*2})^{1/2}$), which describes the brightness or vividness of colour. Three measurements were taken of each sample.

The American Association of Cereal Chemists International (AACC) methods were used for the determination of moisture (method 44-19) and protein (method 46-12) contents of the SLF samples (AACC, 1990). Ash contents of the samples were measured according to ICC (International Association for Cereal Science and Technology) method 104-1 (ICC, 2002).

The mineral contents of WF, lupin flour and SLF flour were determined by inductively coupled plasma spectroscopy (ICP-AES, Vista series; Varian International AG, Cham, Switzerland). Dry samples were digested using a closed vessel microwave digestion oven (MARS 5; CEM Corporation, Matthews, NC, USA) with concentrated nitric acid and sulphuric acid. Concentrations were determined by ICP-AES (Bubert and Hagenah, 1987).

PA was measured by a colorimetric method according to Haugh and Lantzsch (1983). PA in the sample was extracted with a solution of HCl (0.2 N) and precipitated with a solution of ammonium iron (III) sulphate ($NH_4Fe(SO_4)_2 \cdot 12H_2O$).

Statistical analyses

JMP statistical package software (version 5.0.1; SAS Institute. Inc., Cary, NC, USA) was used to perform the statistical analyses. Data were assessed by analysis of variance. Tukey HSD test was used to separate means. Significance was accepted at $P<0.05$ throughout the analysis.

3. Results and discussion

Chemical properties

Table 1 lists the results for the ash, protein and mineral content of WF, NSLF and 1, 3 and 6 day SLF samples. The lowest ash content was obtained with WF, the ash content of lupin samples increased with the sprouting period. The same upward trend was found in protein content. Sprouting brought a slight, but significant ($P<0.05$) rise in protein content. Upward trends in ash and protein have also been identified by other researchers (Gulewicz *et al.*, 2008; Obeidat *et al.*, 2013). The highest protein content was found with 6 day SLF.

As shown in Table 1, the PA content of the WF and NSLF were 349.58 and 1,426.55 mg/100 g, respectively. During the sprouting period, the PA content decreased because of its hydrolysis by the phytase enzyme. Sokrab *et al.* (2012) also reported that germination significantly reduces the PA content of grains with time. Additionally, a significant decrease (36.5%) in PA occurs in lupin seeds during the first 48 h of germination as has been shown by Muzquiz *et al.* (1998). A reduction ratio in PA after 6 days sprouting process was found as 88.97% which is very important for the bioavailability of mineral matter in SLF. Bartnik and Szafranska (1987) reported that phytase activities increased, and on the contrary phytate contents in cereals decreased during sprouting. Therefore, sprouting or germination processes have been widely used for their ability to minimise the levels of antinutritional factors present in legume seeds, and also improve the concentration and bioavailability of their nutrients (Larsson and Sandberg, 1992).

The mineral contents (calcium, Ca; copper, Cu; potassium, K; magnesium, Mg; manganese, Mn; phosphorus, P; and zinc, Zn) of WF, lupin flour and SLF are presented in Table 1. K, Mg, Mn, P and Zn contents of the SLF samples increased except for Ca with increasing sprouting period. Ca contents of sprouted lupin samples showed a downward trend. There is no significant ($P<0.05$) difference for Cu content between NSLF and SLF samples. The K, Mn, P and Zn contents increased during the sprouting period. The increment in these elements contents may be due to hydrolysis of PA by the enzyme phytase, which is released during sprouting. Sokrab *et al.* (2012) reported that during the sprouting time sodium (Na), K, Mg, Mn, Cu, cobalt (Co), P, Zn and iron (Fe) contents of sprouted corn seeds increased and also the extractability of minerals of nutritional importance (Ca and P) significantly ($P<0.05$) increased with the sprouting time.

In this study, the Mn content of SLF increased with sprouting period up to 103.70 mg/100 g and 154.77 times compared to WF. Mn^{2+} ions function as cofactors for a large

Table 1. Some chemical properties of wheat flour (WF), non-sprouted lupin flour (NSLF) and sprouted lupin flour (SLF).[1]

	WF	NSLF	1 day SLF	3 day SLF	6 day SLF
Ash (g/100 g)	0.51±0.01e	3.57±0.03d	3.66±0.02c	3.74±0.03b	3.82±0.01a
Protein[2] (g/100 g)	12.2±0.19e	30.30±0.14d	31.53±0.06c	32.59±0.13b	33.30±0.09a
Calcium (mg/100 g)	24.5±0.61c	101.78±0.80a	81.83±0.89b	77.26±1.95b	74.17±2.56b
Copper (mg/100 g)	39.6±0.74a	0.65±0.02b	0.67±0.02b	0.67±0.00b	0.68±0.01b
Potassium (mg/100 g)	164.94±1.00d	991.57±1.54c	1010.19±0.94b	1015.29±0.54b	1025.68±1.85a
Magnesium (mg/100 g)	39.6±3.83b	126.85±0.44a	131.69±1.99a	133.19±1.68a	133.32±0.88a
Manganese (mg/100 g)	0.67±0.04c	95.34±0.07b	102.24±1.59a	102.95±0.54a	103.70±2.10a
Phosphorus (mg/100 g)	134.6±0.96d	410.99±1.87c	447.84±1.99b	480.97±0.53a	486.04±1.30a
Zinc (mg/100 g)	1.23±0.04d	4.45±0.05c	4.73±0.03bc	4.83±0.04ab	4.96±0.02a
Phytic acid (mg/100 g)	349.58±21.20c	1426.55±21.51a	1368.76±8.60a	997.67±17.20b	157.26±15.92d

[1] Variables were determined by the one-way ANOVA model; means followed by different letters in the same rows are significantly different ($P<0.05$).
[2] Protein for lupin seeds = N×6.25; for wheat flour = N×5.70.

variety of enzymes. Mn is an essential element for human health, fatness, glucose intolerance, blood clotting, skin problems, lowered cholesterol levels, skeleton disorders, birth defects, changes of hair colour and neurological symptoms (http://tinyurl.com/26xxdj). But exposure to high concentrations of Mn can be detrimental to health (www.epa.gov/teach/).

Colour

Colour values of WF, NSLF and SLF are shown in Table 2. WF gave the highest lightness (L*) value. The L* value of SLF significantly decreased during the sprouting. WF and lupin flour presented similar a* values (-0.48 and -0.48, respectively). After the 6 days period of sprouting, a* values of SLF gave higher redness values (-0.66) than 3 day SLF. Sprouting showed significant (P<0.05) increase in b* values. Shin *et al.* (2013) showed that 2 day germinated soy flour gave lower a*, b*, chroma values than raw soya bean flour and hue angle values of soya bean flour increased with germination. The mean value of hue angle and chroma are listed in Table 2. The hue angle values of flours varied from 91.03 to 92.94. This parameter was not affected by the sprouting. The chroma values were found to increase from 26.41 to 33.61 with an increase in sprouting period from 0 to 6 days.

Technological properties

Table 3 shows the loaf weight, volume and specific volumes of bread samples. Changes in loaf weight values were not significant at the P<0.05 level. 1 and 3 day SLF bread showed 520.0 and 572.5 ml bread volume, which were higher than the other bread samples. The volume and specific volume of bread samples increased up to 3 days of sprouting and thereafter started to decrease. An increase in enzyme activity during sprouting (e.g. amylase) positively affected volume of the breads. A 3 day sprouting period could be the optimum for enzyme activity. A slight decrease in volume of the bread occurred with the 3 day SLF bread but the volume of bread containing 6 day SLF was higher than WF bread sample. Hallen *et al.* (2004) reported that specific volume of germinated flour bread is decreased by 23%, from 5 to 20%, with sprouted cowpea flour substitution. Additionally, Dervas *et al.* (1999) and Doxastakis *et al.* (2002) reported that lupin flour results in a smaller bread volume and has

Table 2. Colour values of wheat flour (WF), non-sprouted lupin flour (NSLF) and sprouted lupin flour (SLF).[1]

Flours	L*	a*	b*	Hue angle	Chroma
WF	93.47±0.20a	-0.48±0.06a	9.62±0.38d	92.85±0.49a	9.63±0.38d
NSLF	88.02±0.18b	-0.48±0.08a	26.41±0.27c	91.03±0.18a	26.41±0.27c
1 day SLF	87.34±0.05b	-0.92±0.08a	29.77±0.11b	91.76±0.14a	29.78±0.12b
3 day SLF	85.71±0.10c	-1.60±0.16b	31.14±0.45ab	92.94±0.34a	31.16±0.44ab
6 day SLF	81.02±0.09d	-0.66±0.04a	33.61±0.69a	91.12±0.04a	33.61±0.69a

[1] Tukey HSD test; means followed by the same letter within a column are not significantly different (P<0.05). Values are the average of triplicate measurements of the duplicate sample ± standard deviation.
L* = lightness/darkness (100 = white; 0 = black); a* = redness/greenness (+ = red; - = green); b* = yellowness/blueness (+ = yellow; - = blue).

Table 3. Effects of sprouted lupin flour (SLF) on bread properties.[1]

Breads	Weight (g)	Volume (ml)	Specific volume (ml/g)	Symmetry (1-5)	Texture (1-5)	Porosity (1-5)	Firmness (N/cm²)	
							1st day	3rd day
Wheat flour	147.30±0.28a	425.0±21.21b	2.89±0.15bc	3.60±0.14c	4.55±0.07a	3.90±0.14a	0.28±0.03a	0.43±0.02a
Non-sprouted lupin flour	149.45±0.07a	412.5±3.54b	2.51±0.09c	4.05±0.07bc	3.60±0.14bc	5.00±0.00a	0.36±0.03a	0.50±0.03a
1 day SLF	148.70±2.40a	520.0±14.14a	3.50±0.15ab	4.60±0.14ab	3.90±0.14b	4.65±0.21a	0.33±0.00a	0.46±0.00a
3 day SLF	148.20±0.14a	572.5±10.61a	3.85±0.09a	4.95±0.07a	5.00±0.00a	3.90±0.14a	0.32±0.04a	0.45±0.03a
6 day SLF	148.70±0.28a	507.5±17.68ab	3.41±0.12abc	2.95±0.07d	2.90±0.14c	3.00±0.00b	0.26±0.01a	0.42±0.01a

[1] Tukey HSD test; means followed by the same letter within a column are not significantly different (P<0.05). Values are the average of triplicate measurements of the duplicate sample ± standard deviation.

negative effects on quality attributes, such as crumb grain and tenderness. But sprouting processes of lupin flour improve bread volume compare to NSLF.

Table 3 shows the symmetry, texture, porosity and firmness values of the breads. Breads containing 3 day SLF gave the highest symmetry values in all bread samples. The highest texture values obtained with 3 day SLF bread, also the control bread sample showed statistically similar effects on texture. WF, NSLF, 1 day and 3 day SLF produced bread with smallest pores, but the 6 day SLF with the biggest pores. In another study, cake produced with 30% lupin flour addition gave higher symmetry index values than control cake sample (Levent and Bilgiçli, 2011).

Firmness values are shown in Table 3. Comparison of the means of the different sprouting periods showed that firmness on the first and third day, for all the bread samples gave the same statistically classification. 10% SLF addition does not affect the firmness values. In a study by Obeidat *et al.* (2013) it was shown that up to 5% substitution of WF with germinated lupin flour produced acceptable cookies. Paraskevopoulou *et al.* (2010) reported that the incorporation of lupin protein isolates to WF delayed bread firming. Ziobro *et al.* (2013) defined that addition of lupin proteins significantly modified colour and textural properties of bread crumb and decreased hardness and chewiness of the bread crumb compared to the control sample.

Crust and crumb colour of breads produced with WF, lupin flour and SLF were shown in Table 4. The highest L* crust colour values of breads were obtained with 3 day SLF lupin breads. There were no significant differences ($P<0.05$) between the lupin flour breads (sprouted and non-sprouted) for the a* parameter. The lowest lightness and highest redness of crumb colour were obtained with 6 day SLF breads. In the study of Levent and Bilgiçli (2011), crust and crumb lightness of the gluten-free cakes decreased with all levels of lupin flour addition compared to a control sample. Lupin flour (sprouted and non-sprouted) addition in bread making showed more yellowish crumb colour compared to the control sample. In other research, breads substituted with lupin flour in place of WF, had a dark crust colour and more yellowish crumb colour and thickened cell crumb texture (Dervas *et al.*, 1999; Doxastakis *et al.*, 2002, 2007; Pollard *et al.*, 2002). This darkening of the SLF bread crust might have been attributed to Maillard reactions during baking due to the high lysine and free sugar content of lupin. Hefni and Witthöft (2011) reported that with the replacement of flour with sprouted WF, the bread was dark and layers were not separated. Also Hallen *et al.* (2004) determined that the crust and crumb colour of breads became progressively darker in bread contained sprouted cowpea flour.

4. Conclusions

The effect of SLF on the quality properties of bread was investigated. Compared to WF, the ash and protein content of SLF increased with sprouting from a 1 to 6 days period. During sprouting, PA hydrolysed by the phytase enzyme, and the PA content was decreased. Mineral (K, Mn, P and Zn) contents of the SLF samples increased with increasing sprouting period. Lupin flour addition did not affect firmness of bread samples significantly. 6 day SLF bread samples had the biggest crumb pores, but NSLF, 1 day and 3 day SLF bread dough had similar pore size (the smallest) compared to the control bread sample. As a result, breads could be enriched functionally and nutritionally with 3 day SLF addition at the 10% level without any adverse effect on the quality properties of bread. The sprouting process increased both mineral amount and also the mineral

Table 4. Effect of sprouted lupin flour (SLF) on crumb and crust colour of breads.[1]

Breads	Crust			Crumb		
	L*	a*	b*	L*	a*	b*
Wheat flour	57.52±2.04a	10.34±0.41a	20.67±2.69a	70.29±0.88a	-1.30±0.06b	10.42±0.32b
Non-sprouted lupin flour	46.01±1.46b	11.94±0.71a	14.20±1.47a	68.01±0.95ab	-1.58±0.28bc	19.26±0.18a
1 day SLF	47.42±1.48b	12.05±0.68a	16.34±1.55a	64.95±1.13ab	-1.62±0.07bc	19.36±0.08a
3 day SLF	56.40±0.65a	12.38±0.08a	24.89±1.36a	63.17±0.47bc	-1.75±0.02c	19.57±0.40a
6 day SLF	48.32±0.36b	12.92±0.68a	18.19±0.73a	57.84±1.61c	-0.58±0.11a	19.73±0.50a

[1] Tukey HSD test; means followed by the same letter within a column are not significantly different ($P<0.05$). Values are the average of triplicate measurements of the duplicate sample ± standard deviation.
L* = lightness/darkness (100 = white; 0 = black); a* = redness/greenness (+ = red; - = green); b* = yellowness/blueness (+ = yellow; - = blue).

bioavailability by means of releasing some minerals with destructing of PA.

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