Influence of Chemical Properties of Wheat-Lupine Flour Blends on Cake Quality

Abdelrahman R. Ahmed[1,2,*]

[1]Faculty of Specific Education, Home Economics Department, Ain Shams University, Cairo, Egypt
[2]Institute for Food Technology and Food Chemistry, Department of Food Rheology, Technical University of Berlin, Sekr, KL-H1, Königin-Luise-Str, Berlin / Germany
*Corresponding author: abdohat1@yahoo.com

Abstract   Legumes have interesting nutritional properties and their inclusion in diet is encouraged; their incorporation into bakery products could be a good method for increasing consumption. In this study, the influence of the partial replacement of wheat flour by lupine flour on the quality characteristics of butter cake was analyzed. In the present study, three different concentrations (5, 10 and 15 % as a substitution of wheat flour) of lupine flour were used. Lupine flour showed higher levels of moisture, crude protein, ash, crude fat and dietary fiber than the wheat flour. The lupine flour showed higher levels of phenolic and flavonoids than the wheat flour. Conversely, wheat flour showed higher levels of total flavonols. Results clearly indicate that lupine flour exhibited higher antioxidant activity with DPPH and ABTS than the wheat flour. Essential amino acids (lysine, threonine, isoleucine, phenylalanine and tryptophane) in lupine flour were higher than those in wheat flour except methionine content which was higher in wheat flour (1.7 g/kg). A sensory acceptability of the cake is satisfactory up to 10 % concentration of lupine flour given. Even though deterioration in the structural formation in the batter system after the addition of lupine flour were detected that the blends have relatively good structure due to dominance of wheat to bake acceptable, protein enriched consumable cake. Finally, lupine flour up to 10% can be used successfully in bakery products. This could be utilized for the development of composite blends from locally produced lupine at small scale industry level as value-add products.

Keywords: lupine, phenolic compounds, amino acids, cake quality

1. Introduction

Cakes are important bakery products. Their worldwide market currently grows with about 1.5% a year. Challenges in the cake market include cost reduction, increased shelf life and quality control. Cake making consists of mixing the ingredients into a batter which, because of the high level of liquid phase in cake recipes, has a low viscosity, and baking such batter into cake [1].

Wheat-flours used in cake elaboration have lower protein content, and it is known that one of the most important characteristics of cake elaboration flours is particle size [2]. Gluten does not play an important role in this kind of product, which means that flours from other cereals [3,4,5] or even from pulses, such as chickpeas or lupine [6,7] can be used.

Pulses, also known as grain legumes, are essential to human diets in many parts of the world [8]. Pulses are unique for a human diet in terms of their nutritional profile. They are rich in protein, carbohydrates, dietary fiber, some minerals and vitamins and they are also low in fat [9,10,11].

Legume/pulse proteins, because of their composition, are considered a good supplement for cereal based foods, since both legume and cereal proteins are complementary with regard to their essential amino acids. Legume proteins are rich in lysine and deficient in sulphur-containing amino acids, whereas cereal proteins are deficient in lysine, but have adequate amounts of sulphur amino acids [12]. Apart from being nutritious, pulse proteins are highly functional and exhibit properties like solubility, gelation and water binding playing a crucial role in structure formation and mouth feel of the finished products. Among the legumes/ pulses tested as protein-enriching agents of bakery products, in the form of various protein preparations (e.g. flour, protein isolate, etc.), are soybean, chickpea, pea and lupine [6,13,14,15,16,17].

Research has indicated that consumption of pulses may have potential health benefits including reduced risk of cardiovascular disease, cancer, type-2 diabetes, osteoporosis, hypertension, gastrointestinal disorders, adrenal disease and reduction of LDL cholesterol [18-26].

The consumption could be higher if the food industry and professional organizations take up the challenge to incorporate grain legumes in novel, convenient and healthy food products [27]. Because of the nutritional and

health promoting properties of the pulses, the development of value-added pulse based products for new market opportunities in the functional food and nutraceutical industry is being promoted [28].

Lupine flour is a novel food ingredient derived from the endosperm of lupine, a grain legume. It contains 40–45 % protein, 25–30 % fiber, and negligible sugar and starch [29]. It can be incorporated into high carbohydrate foods, resulting in significant increases in protein and fiber, reductions in refined carbohydrate, and little change in product acceptability [30]. Increasing protein at the expense of refined carbohydrate in the diet may benefit blood pressure. Therefore, the aim of this study was to determine the effect of the partial replacement of wheat flour by lupine flour on the quality of cakes.

2. Materials and Methods

2.1. Materials

Local Egyptian breeds of lupine (*Lupinus albus L.* variety Giza) were obtained from the Agricultural Research Centre, Giza, Egypt. Lupine flour was obtained after grinding lupine grains in a laboratory hammer mill (Retsch - Germany) until they could pass through a 250 µm screen. Commercial wheat flour type 405 was obtained from Lidl Market (Berlin-Germany). All other chemical reagents used in the experimental analysis were of analytical grade.

2.2. Chemical Analysis

2.2.1. Proximate Composition

Proximate composition was carried out according to ICC Standard Methods [31]. Moisture content was determined by drying the samples at 105°C to constant weight (ICC 109/01). Ash content was determined by calcinations at 900°C (ICC 104/1). Nitrogen content was determined by using Kieldahl method with factor of 5.7 to determine protein content (ICC 105/2). The total lipid content was determined by defeating in the Soxhelt apparatus with hexane (ICC 136). The determination of starch content was assessed using a polarimetric method according to Ewers, modified by [32]. All the measurements of analyzed samples were made in triplicate.

2.2.2. Determination of Total Phenolics

Total phenolic content was determined by the Folin–Ciocalteu micro-method [33]. A 20 µL aliquot of extract solution was mixed with 1.16 mL of distilled water and 100 µL of Folin–Ciocalteu's reagent followed by 300 µL of 200 g L^{-1} Na_2CO_3 solution. The mixture was incubated in a shaking incubator at 40°C for 30 min and its absorbance at 760 nm was measured. Gallic acid was used as standard for the calibration curve. Total phenolic content expressed as gallic acid equivalent (GAE) was calculated using the following linear equation based on the calibration curve:

$$Ab = 0.98C + 9.925 * 10^{-3} \left(R^2 = 0.9996 \right) \quad (1)$$

where Ab is the absorbance and C is the concentration (mg GAE g^{-1} dry weight (DW)).

2.2.3. Determination of Total Flavonoids

Total flavonoid content was determined by the method of Ordoñez et al., [34]. A 0.5 mL aliquot of 20 g L^{-1} $AlCl_3$ ethanolic solution was added to 0.5 ml of extract solution. After 1 h at room temperature the absorbance at 420 nm was measured. A yellow color indicated the presence of flavonoids. Extract samples were evaluated at a final concentration of 0.1 mg ml^{-1}. Total flavonoid content expressed as quercetin equivalent (QE) was calculated using the following equation based on the calibration curve:

$$C = 0.0255 * Ab \left(R^2 = 0.9812 \right) \quad (2)$$

where Ab is the absorbance and C is the concentration (mg QE g^{-1} DW).

2.2.4. Determination of Total Flavonols

Total flavonol content was determined by the method of Kumaran and Joel Karunakaran, [35]. To 2 mL of extract solution, 2 mL of 20 g L^{-1} $AlCl_3$ ethanolic solution and 3 mL of 50 g L^{-1} sodium acetate solution were added. The absorption at 440 nm was read after 2.5 h at 20°C. Extract samples were evaluated at a final concentration of 0.1mg mL^{-1}. Total flavonol content expressed as QE was calculated using the same equation of flavonoids.

2.3. Antioxidant Activity of Extracts

Because of the differences among the various test systems available, the results of a single method can provide only a limited assessment of the antioxidant properties of a substance [36]. For that reason, in this study the antioxidant capacity of each extract was determined through two complementary assay procedures.

2.3.1. DPPH Radical-Scavenging Activity

The DPPH assay according to Lee et al., [37] was utilised with some modifications. The stock reagent solution (1 x 10^{-3} mol L^{-1}) was prepared by dissolving 22 mg of DPPH in 50 mL of methanol and stored at −20°C until use. The working solution (6 x 10^{-5} mol L^{-1}) was prepared by mixing 6 mL of stock solution with 100 mL of methanol to obtain an absorbance value of 0.8 ± 0.02 at 515 nm, as measured using a spectrophotometer. Extract solutions of different concentrations (0.1 mL of each) were vortexed for 30 s with 3.9 mL of DPPH solution and left to react for 30 min, after which the absorbance at 515 nm was recorded. A control with no added extract was also analysed. Scavenging activity was calculated as follows:

$$\text{DPPH radical} - \text{scavenging activity} \left(\% \right)$$
$$= \left[\left(Ab_{\text{control}} - Ab_{\text{sample}} \right) / Ab_{\text{control}} \right] * 100 \quad (3)$$

where Ab is the absorbance at 515 nm.

2.3.2. ABTS Radical-Scavenging Activity

For the ABTS assay the method of Re et al., [38] was adopted. The stock solutions were 7 mmol L^{-1} ABTS solution and 2.4 mmol L^{-1} potassium persulfate solution. The working solution was prepared by mixing the two stock solutions in equal quantities and allowing them to

react for 12–16 h at room temperature in the dark. Then 1 mL of the resulting ABTS$^+$ solution was diluted with 60 mL of methanol to obtain an absorbance of 0.706 ± 0.001 units at 734 nm, as measured using a spectrophotometer. ABTS$^+$ solution was freshly prepared for each assay. Extract solutions of different concentrations (1 mL of each) were allowed to react with 1 mL of ABTS$^+$ solution for 7 min, after which the absorbance at 734 nm was recorded. A control with no added extract was also analysed. Scavenging activity was calculated as follows:

$$\begin{aligned} &\text{ABTS radical} - \text{scavenging activity } (\%) \\ &= \left[\left(\text{Ab}_{control} - \text{Ab}_{sample} \right) / \text{Ab}_{control} \right] * 100 \end{aligned} \quad (4)$$

where $\text{Ab}_{control}$ is the absorbance of ABTS radical + methanol Ab_{sample} is the absorbance of ABTS radical + extract.

2.4. HPLC Analysis

A total of 20 mg grounded dried samples were extracted for 15 min using 750 µL 70 % methanol (v/v, pH 4, phosphoric acid) in an ultrasonic water bath (Sonorex digital 10 p, Bandelin) on ice. Samples were centrifuged for 5 min at 6000 rpm. The supernatants were collected and the pellets were re-extracted twice more with 500 µL 70 % methanol. The combined supernatants from each sample were reduced to near dryness in a centrifugation evaporator (Speed Vac, SC 110) at 25°C. Samples were then made up to 1 ml with 40 % acetonitrile. The samples were filtrated using 0.22 µm filter, and then analyzed with HPLC (Dionex Summit P680A HPLC-System), equipped with P680 pump, ASI-100 automated sample injector, a Narrow-Bore Acclaim PA C16-column (3 µm, 2.1 * 150 mm, Dionex) and PSA-100 photodiode array detector (Dionex) and software Chromeleon 6.8 (Dionex, USA). The column was operated at a temperature of 35°C.

The mobile phase consisted of 0.1 % (v/v) phosphoric acid in ultrapure water (eluent A) and of 40 % (v/v) acetonitrile in ultrapure water (eluent B). A multistep gradient was used for all separations with an initial injection volume of 40 µL and a flow rate of 0.4 mL/min. The multistep gradient was as follows: 1 min: 0.5 % (v/v) B; 1-10 min: 0.5-40 % B; 10-12 min: 40 % B; 12-18 min: 40-80 % B; 18-20 min: 80 % B; 20-24 min: 80-99 % B; 24-30 min: 99- 100 % B; 30-34 min: 100-0.5 % B; 34-39 min: 0.5 % B. Simultaneous monitoring was performed at 290, 330 and 254 nm at a flow rate of 0.4 mL/min. Phenolic acid quantity was calculated from HPLC peak areas at 290 nm against the internal standard and external standards. Identification and quantification of phenolic acids present was done by comparing retention time and area of the peaks in the extracts with that of the standard phenolic acids (chlorogenic acid, caffeic acid, cinnamic acid, coumaric acid, rosmarinic acid and sinapic acid).

2.5. Amino Acid Analysis

Amino acid content was determined as described by Moore et al., [39]. The analysis was performed in Central Service Unit, National Research Centre, Egypt using LC3000 amino-acid analyzer (Eppendorf-Biotronik, Germany). The technique was based on the separation of the amino acids using strong cation exchange chromatography followed by the ninhydrin colour reaction and photometric detection at 570 nm. Standard amino acids were used for comparison of resulting profiles, allowing quantitation of amino-acid residues. The defatted powdered seeds were hydrolyzed with 6 N HCl at 110°C in teflon capped vials for 24 h. After vacuum removal of HCl, the residues were dissolved in a lithium citrate buffer, pH 2.2. Twenty µL of the solution were loaded into the cation exchange column (pre-equilibrated with the same buffer), then four lithium citrate buffers with pH values of 2.2, 2.8, 3.3 and 3.7, respectively, were successively applied to the column at a flow rate of 20 mL/min. The ninhydrin flow rate was 10 mL/h under these conditions and a typical analysis required 160 min.

Methionine was determined as methionine sulfone, after oxidation with performic acid. An amino acid standards containing cysteine were treated parallel with the samples and used to quantify the methionine content. The amino acid content of the reference protein was taken from [40].

2.6. Cake Formulation and Baking

The recipes of butter cake used: 100 g (wheat flour or wheat flour substituted with 5, 10 or 15 % lupine flour), 80 g egg, 60 g sugar, 50 ml Milk (1.5 % fat), 50 g margarine and 4 g baking powder. A creaming mixing procedure was used. All ingredients, except for the flour and baking powder, were mixed for 2 min at speed 6 using a Kitchene Aid Professional mixer (KPM5). After the addition of the flour and baking powder, the mixing process continued for 3 min at speed 8. 200 g of cake batter were placed into 120 mm diameter and 45 mm height, metallic, lard coated pan, and were baked in an electric oven for 25 min at 200°C.

2.7. Loaf Volume Determination

Cake mass was weighted after 3 hours at room temperature. The volume (cm^3) was measured by rapeseed replacement method described in the [41]. The specific volume was obtained by dividing the volume by their weights.

2.8. Color Measurements

Crust and crumb color of fresh cake was measured with a Minolta Calorimeter (CR 200 Japan). Color readings were expressed by Hunter values for L*, a* and b*.

2.9. Sensory Evaluation

Evaluation of the cake quality characteristics was carried out following cooling to room temperature for 2 h. Sensory evaluation was performed by ten trained panelists who were graduate students and staff members of the Department of Rheology, Institute of Food Technology and Food Chemistry, Technical University, Berlin. Cakes were randomly assigned to each panelist. The panelists were asked to evaluate each sample for appearance, crumb texture, crumb grain, crust color, taste, odor and overall acceptability. A 10 point scale was used where 10 "excellent and 1" extremely unsatisfactory.

2.10. Statistical Analysis

Analysis of variance (ANOVA) was carried out using SAS program (Statistical Analysis System version. 9.1) SAS Institute Inc. [42]. The chemical composition and cake characteristics of wheat batter with or without lupine flour were analysed using ANOVA. When the treatment factor effect was found significant, indicated by a significant F-test ($p < 0.05$), differences between the respective means were determined using least significant difference (LSD) and considered significant when $p < 0.05$. Mean ± standard deviation of mean was used.

3. Results and Discussion

3.1. Chemical Composition of Wheat Flour, Lupine Flour and Their Blends

Lupine is a good source of nutrients, not only proteins but also lipids, dietary fiber, minerals, and vitamins [43]. The results for the chemical composition of wheat flour (WF), lupine flour (LF), and their blends are shown in Table 1. The lupine flour showed higher levels of moisture, crude protein, ash, crude fat and dietary fiber than the wheat flour. Conversely, wheat flour showed higher levels of starch. Highly significant differences ($P < 0.05$) were observed between the two types of flours. Mean protein and dietary fiber increased with increasing amount of lupine flour added. There was no significant difference between wheat flour and supplemented flour with different concentration of lupine for moisture content (Table 1).

The chemical properties of wheat flours have been studied previously by several researchers and they found that moisture content ranged between 12.5 to 14.6 % crude protein content 8.23 to 12.71 % and ash content 0.42 to 0.66 [44].

Table 1. Chemical composition of wheat flour (WF), lupine flour (LF) and their blends

Analysis	WF	LF	LF level (%)		
			5	10	15
Moisture	11.27 ± 0.09	12.37 ± 0.46	11.35 ± 0.19	11.42 ± 0.17	11.54 ± 1.16
Protein	12.1 ± 0.20	38.6 ± 0.87	13.73 ± 0.24	14.75 ± 0.27	16.28 ± 0.31
Ash	0.40 ± 0.02	3.41 ± 0.03	0.55 ± 0.01	0.70 ± 0.04	0.85 ± 0.03
Fat	1.62 ± 0.19	9.94 ± 0.16	2.04 ± 0.19	2.45 ± 0.17	2.87 ± 0.16
Starch	69.8 ± 1.96	0.98 ± 0.04	66.36 ± 3.94	62.92 ± 1.88	59.48 ± 2.84
S. D. F	1.1 ± 0.09	11.0 ± 1.36	1.60 ± 0.14	2.09 ± 0.27	2.59 ± 0.31
I. D. F	1.6 ± 0.23	30.8 ± 2.45	3.06 ± 0.31	4.52 ± 0.42	5.98 ± 0.53
T. D. F	2.7 ± 0.15	41.8 ± 3.08	4.66 ± 0.27	6.61 ± 0.43	8.57 ± 0.60

Mean ± standard deviation. S.D.F: Soluble Dietary Fiber. I.D.F: Insoluble Dietary Fiber. T.D.F: Total Dietary Fiber.

Table 2. Extract yield, total polyphenols content and antioxidant capacity of wheat flour (WF), lupine flour (LF) and their blends

Analysis	WF	LF	LF level (%)		
			5	10	15
Yield extract (%)	13.7 ± 3.99	36.2 ± 1.73	11.9 ± 0.35	13.6 ± 1.35	14.0 ± 1.21
Total phenolic (µg GAE/g DW)	126.63 ± 3.52	138.17 ± 8.35	132.17 ± 0.58	142.5 ± 7.10	156.53 ± 3.88
Total flavonoids (µg QE/g DW)	6.33 ± 0.15	8.93 ± 0.06	7.67 ± 1.27	7.93 ± 0.06	8.4 ± 0.52
Total flavonols (µg QE/g DW)	32.03 ± 6.13	31.60 ± 4.70	29.10 ± 2.48[*]	28.27 ± 2.96[*]	27.00 ± 1.08[*]
DPPH (%)	3.31 ± 0.35	20.62 ± 1.22	5.1 ± 0.10[*]	6.04 ± 0.77[*]	7.16 ± 0.26[*]
ABTS (%)	26.7 ± 0.21	43.42 ± 0.37	29.41 ± 0.37[*]	31.09 ± 0.00[*]	32.35 ± 0.37[*]

Mean ± standard deviation

Protein content of lupine (38.6 %) was higher than that of a lot of legumes. Favier et al., [45] reported that haricot bean, lentil and soy bean contain 28.8 %, 26.7 % and 40.5 % protein, respectively. Because of the high protein content, lupine flour could be used in the human diet. Also, temperature of denaturation of these proteins is higher than animal protein, so they are technologically easier to handle [46]. Lupine flour had a high amount of crude fiber (16.2 %). These fibers have many desirable properties, including white color, high water-holding capacity (7.1 g H_2O/g) and beneficial effects on human health [47]. Therefore, lupine flour can be incorporated into a wide range of foods to make dietary products.

3.2. Phenolic Compounds and Antioxidants Capacity of Wheat Flour, Lupine Flour and Their Blends

Phenolic compounds ubiquitous in plants are key phytochemical drivers of the health and functional foods and nutraceutical industry. Research with polyphenol compounds from various crops has created a growing

market for polyphenol-rich ingredients, estimated to be worth around $ 99 million in Europe in 2003 [48].

Conventional solvent extraction has been reported in a laboratory scale using acetone, hexane, methanol and ethanol [49]. In this study, methanol was used for the extraction of antioxidant compounds from wheat, lupine flour and their blends (Table 2). The extraction yield 13.7 and 36.2 g/100 g dry weight for wheat and lupine flour respectively.

The lupine flour showed higher levels of total phenolic and total flavonoids than the wheat flour. Conversely, wheat flour showed higher levels of total flavonols. These results confirmed by statistical analysis, which highly significant differences ($P<0.05$) were observed between the two type of flours. Total phenolic and total flavonoids increased with increasing amount of lupine flour added to be 132.17 ± 0.58, 142.5 ± 7.10, 156.53 ± 3.88 (µg GAE/g DW) and 7.67 ± 1.27, 7.93 ± 0.06, 8.4 ± 0.52 (µg QE/g DW) for substituting wheat flour with lupine flour at 5, 10 and 15 %, respectively on dry weight basis. The contents of phenolic acids in lupine used in this study are comparable to levels reported previously [50], especially

in cultivars of L. albus grown in Portugal. Phenolic content of lupins were higher than those of bean cultivars grown in Manitoba [51] probably as a result of relatively high flavonoid content. The methanolic extracts of lupine seed were analysed by high performance liquid chromatography figure 15.

As shown in figure 1, nine phenolic acids were separated and identified. This method is well reproducible and provides good separation in terms of migration time and resolution.

The antioxidant effects of extracts of various wheat flour (WF), lupine flour (LF) and their blends at different concentration (5, 10 and 15 %) were measured. Since the active substances of flour extracts tested are different, the antioxidant activities of these extracts cannot be evaluated by only a single method. Therefore, two different models were used in this study [52].

Free radicals which are involved in the process of lipid peroxidation are considered to play a major role in numerous chronic pathologies, such as cancer and cardiovascular diseases among others [53]. The DPPH radical has been widely used to evaluate the free radicals' scavenging ability of various natural products and has been accepted as a model compound for free radicals originating in lipids [54]. The effect of antioxidants on diphenyl-p-picryl hydrazyl (DPPH) radical scavenging was thought to be due to their hydrogen donating ability. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule.

The assay is based on the reduction of DPPH. Because of its odd electron, DPPH gives strong absorption maxima at 515 nm (purple color) by visible spectroscopy. As the odd electron of the radical becomes paired off in the presence of a hydrogen donor, i.e., a free radical scavenging antioxidant, the absorption intensity is decreased, and the resulting decolorization is stochiometric with respect to the number of electrons captured [55].

Table 2 showed that the scavenging activity of methanolic extracts against DPPH for wheat flour (WF), lupine flour (LF) and their blends. Significant ($p < 0.05$) differences between wheat and lupine flour extracts were observed. Results clearly indicate that lupine flour exhibited higher antioxidant activity with DPPH and ABTS than the wheat flour. The antioxidant activity increased with increasing amount of lupine flour added to be 5.1 ± 0.10, 6.04 ± 0.77, 7.16 ± 0.26 in DPPH and 29.41 ± 0.37, 31.09 ± 0.00, 32.35 ± 0.37 in ABTS respectively, for substituting wheat flour with lupine flour at 5, 10 and 15 %, respectively on dry weight basis.

Wang et al., [56] found that some compounds which have ABTS[+] scavenging activity did not show DPPH scavenging activity. In this study, there was not the case. The ABTS[+] scavenging data suggests that the components within the extracts are capable of scavenging free radicals via a mechanism of electron/hydrogen donation and should be able to protect susceptible matrices from free radical-mediated oxidative degradation.
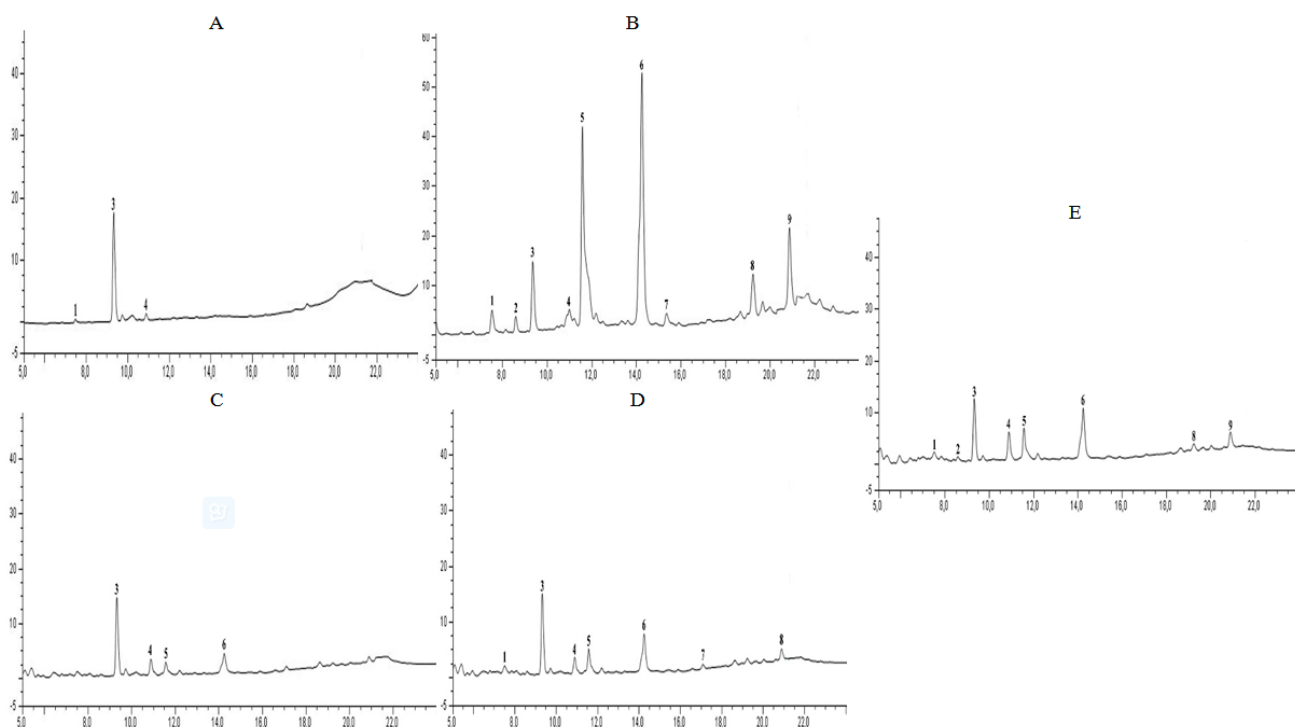

Figure 1. HPLC chromatogram of methanol extract of: wheat flour (A), lupine flour (B) and wheat flour supplemented with lupine flour at different concentration, 5 % (C). 10 % (D) and 15 % (E). 1. gallic, 2. procatechuic, 3. p-hydroxybenzoic, 4. vanillin, 5. P coumaric, 6. chlorogenic, 7. cinnamic 8. sinapine and 9. ferulic acid

3.3. Amino Acids Content

Lupine seeds represent a good balance of essential amino acids [57]. They are considered to be a good source of lysine, and are generally poor in the sulfur-containing amino acids (methionine and cysteín) [58] and threonine [59].

The results for the amino acid content of wheat flour (WF) and lupine flour (LF) are shown in Table 3. In contrast to plants, humans and animals are able to synthesize only 9 amino acids used in protein synthesis

(non-essential amino acids). The biosynthesis of the remaining (essential) amino acids, thereby the protein synthesis, is not possible without their continuous supply through food consumption. In the case of low-protein diets, symptoms, such as delay in growth, negative nitrogen uptake or disturbances in protein synthesis, can take place. Therefore foods rich in exogenous amino acids are desirable.

Table 3. The total amino acids % dry matter, for wheat (WF) and lupine flour (LF)

Amino Acid g/kg	WF	LF	FAO/WHO**	Amino Acid g/kg	FAO/WHO	WF	LF
Lysin*	3,0 ± 0.13	16.35 ± 0.56	5.8	Leucin	6.6	6,7 ± 0.12	26.13 ± 1.08
Threonin*	2,7 ± 0.08	11.48 ± 0.47	3.4	Phenylalanin*	6.3	4,1 ± 0.16	14.20 ± 0.40
Valin	4,8 ± 0.28	16.65 ± 0.76	3.5	Tyrosin	6.1	2,6 ± 0.10	13.90 ± 0.53
Methionin*	1,7 ± 0.15	1.59 ± 0.16	2.5	Tryptophan*	1.1	1,4 ± 0.07	10.94 ± 0.70
Serine	2,3 ± 0.50	1.52 ± 0.60	-	Arginin	-	4,3 ± 0.18	36.13 ± 3.78
Isoleucin*	3,4 ± 0.20	14.85 ± 0.36	2.8	Histidin	1.9	2,5 ± 0.19	5.89 ± 0.33

*Essential Amino Acids **FAO/WHO, [40]

The results showed that the essential amino acids (lysine, threonine, isoleucine, phenylalanine and tryptophane) in lupine flour were higher than those in wheat flour except methionine content which was higher in wheat flour (1.7 g/kg). This result was confirmed by Lubowicki et al., [60]. Sujak et al., [61] reported that lupine seeds of different species representing diverse varieties of sweet lupine grown in Poland manifest a large deficiency of sulphur containing amino acids, for which the recommended level is 3.5 g/16 g N [62]. Methionine levels of 1.59 g/kg, found for the lupine flour was low but comparable to results reported previously for other lupins [63]. The recommended level of methionine is 2.5 g/kg [64]. Of great importance is the presence of sulphur containing amino acids, mainly methionine, which is necessary for the synthesis of cysteine, as well as phenylalanine needed for the synthesis of tyrosine [62].

The protein demand of different organisms depends on their physiological state stipulated mainly by age. For example, young and growing mammals (up to approximately two years in humans) need proteins rich in amino acids, such as arginine and histidine, as such amino acids are the source of the active centers of many enzymes. In contrast, adults show almost no physiological demand for these amino acids.

From the results we can noticed that lupine flour is rich with arginine and histidine (36.13 and 5.89 g/kg respectively). Protein quantity, as well as composition, is the limitation of protein quality [64]. For humans, adequate quantities of lysine, methionine and tryptophan are considered necessary in food of high nutritive value [62]. A number of approaches, based on the analysis of amino acids, have been considered for the estimation of protein quality in human and fodder foods. According to Alsmeyer et al., [65], the nutritional value of food should be expressed in terms of leucine and tyrosine contents, while other classifications are based on the chemical scores for 9–11 amino acids considered essential. Lupine flour showed high content of lysine (16.35 g/kg) more than wheat flour (3.0 g/kg).

3.4. Influence of Lupine Flour Incorporation on Cake Properties

According to Table 4, cake height diminished as the lupine flour percentage increased. During the baking process, baking powder generates gases, which should be retained in order to guarantee good cake volume, and in that respect flour quality has an important role to play. Another important factor is the gelatinization temperature of the flour, as Howard, [66], pointed out for layer cakes, whereas Mizukoshi et al., [67] reached the same conclusion for sponge cakes. The starch gelatinization at low temperatures would prevent the correct expansion of dough.

Table 4. Cake characteristics of wheat flour and lupine flour composite flours

Sample	Cake height cm	Cake weight g	Cake volume cm³	Specific volume cm³/g
WF	7.5 ± 0.53	265.1 ± 13	660 ± 56	2.49 ± 0.6
LF 5 %	7.0 ± 0.33	265.2 ± 38	670 ± 46	2.53 ± 0.45
LF 10 %	6.5 ± 0.23	264.5 ± 11	690 ± 68	2.61 ± 0.27
LF 15 %	6.0 ± 0.65	269.2 ± 8	700 ± 57	2.60 ± 0.30

Mean ± standard deviation of mean.

Lupine flour also presented higher protein content, and different amino acid composition than wheat flours which could affect cake characteristics, especially volume [68]. As far as lupine derivatives are concerned, flour gave cakes with higher volume. This fact can be explained by the higher fiber content of lupine flour and its effect on pasting behavior of starches [69].
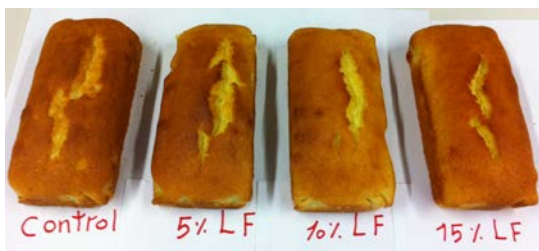

Figure 2. Comparison of the baking properties of wheat flour and mixes with lupine flour cake

The volume index is an indicator of cake volume and, as expected, followed a similar tendency as volume. Symmetry indicates the differences in height between the central zone and the lateral zone. Thus, a high symmetry suggests that cakes mainly rise in their central part, while a negative symmetry indicates that cake volume falls down at the end of the baking process. Hence, symmetry gives an idea about gas retention in the final baking phase. In both cases the incorporation of lupine flour at different levels had no effect on cake symmetry (Figure 2).

This result is not confirmed with Gômez et al., [6] who studied the influence of the total or partial replacement of wheat flour by chickpea flour on the quality characteristics of two kinds of cake. They reported that in both cases the incorporation of chickpea flour reduced their symmetry, and in the case of layer cakes with 100 % of chickpea flour it became negative. As for cake weight, no significant differences were found. Therefore, the water

retention capacity was not affected by the substitution of wheat flour by lupine flour.

Crust color in cakes varied with the quantity and the kind of lupine flour addition. This influence was more important in cakes. The crust color data of cakes are shown in Table 5 Cakes became darker (lower L*) as the lupine flour quantity increased. With regard to the wheat flour, it produced the brightest cakes. No considerable differences in crust yellowness were found among the

different lupine flour samples, while all lupine flour gave higher b values than the control (wheat flour). The crust color of cakes was generated in the baking process due to the Maillard reactions between sugars and amino acids, and the caramelization process of sugars. Therefore, the differences observed when the quantity of lupine flour increased could be attributed to the high protein content and the different amino acid composition of the composite flours compared to the wheat flour.

Table 5. Color measurements of cake from wheat flour and lupine flour composite flours

Color parameters	Crust			Crumb		
	L*	a*	b*	L*	a*	b*
WF	90.53 ± 3.79	1.24 ± 0.69	4.92 ± 2.74	93.62 ± 3.74	0.90 ± 0.60	2.31 ± 1.58
LF 5 %	76.48 ± 8.24	4.19 ± 1.85	11.88 ± 6.20	92.39 ± 1.61	1.32 ± 0.41	2.55 ± 1.59
LF 10 %	74.38 ± 10.43	4.71 ± 2.09	14.22 ± 3.13	92.15 ± 2.31	3.00 ± 0.50	7.78 ± 1.82
LF 15 %	73.44 ± 4.50	4.94 ± 4.05	15.95 ± 1.76	91.48 ± 1.55	4.89 ± 30	11.99 ± 1.73

Mean ± standard deviation of 10 different points on Crust and Crumb.

Figure 3. Crumb color of wheat flour and mixes with lupine flour cake

Table 6. Sensory evaluation of cake from wheat flour and lupine flour composite flours

Sample	Appearance	Crumb texture	Crumb grain	Crust color	Taste	Odor	Overall acceptability
WF	7.9 ± 1.5	7.9 ± 1.5	7.1 ± 1.6	7.6 ± 1.8	7.7 ± 2.1	8.1 ± 1.9	8.2 ± 1.3
LF 5 %	7.7 ± 2.0	8.1 ± 1.1	7.9 ± 0.9	8.3 ± 1.6	7.8 ± 1.8	8.2 ± 1.6	8.1 ± 1.5
LF 10 %	8.0 ± 1.4	8.0 ± 1.5	8.3 ± 0.9	8.1 ± 1.5	7.4 ± 2.2	7.9 ± 1.8	8.1 ± 1.2
LF 15 %	8.6 ± 1.3	7.7 ± 2.1	8.9 ± 1.5	8.7 ± 1.3	8.2 ± 2.0	8.2 ± 1.1	8.7 ± 1.3

Mean ± standard deviation of ten panellists

Cake crumb does not reach temperatures above 100°C, so the Maillard or caramelization reactions by sugars fail to take place. Therefore, crumb color must be the result of the raw materials colors and their interactions. The crumb color data of cakes are shown in Table 5 and Figure 3 Cake crumb of lupine flour samples were lightly darker than control, and the lupine flour addition reduced its luminosity, but this effect was not significant. These results agree with those obtained by Dodok et al., [70], who observed that chickpea flour triggered the change in the crumb color when they studied the addition of chickpea flour to bread dough.

Sensory evaluation results of fortified cake with lupine flour are presented in Table 6. It was found that cake which was fortified with different levels of lupine flour had non significant difference in taste, odour and overall acceptability as compared to unfortified cake (control).

The present results came in agreement with Sabanis et al., [71] who reported that, organoleptic properties (colour, flavour and overall acceptability) improved with a low proportion of chickpea flour, especially for 5 % w/w substitution. Alabi and Anuonye, [72] indicated that up to 50 % of some legume products could be added without significant loss in palatability.

4. Conclusion

This study demonstrates that it is possible to incorporate lupine flour into cake formulations up to 10%. The substitution percentage lupine flour used led to the pursued objectives (nutritional improvement, free-gluten products, special organoleptic characteristics, etc.). This could be utilized for the development of composite blends from locally produced lupine at small scale industry level as value-add products.

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