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# Effective detoxification and decoloration of *Lupinus mutabilis* seed derivatives, and effect of these derivatives on bread quality and acceptance

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## Abstract

**BACKGROUND: A study was done to develop procedures for detoxifying *Lupinus mutabilis* seeds, and decreasing or eliminating yellow colour in derivatives from them. An evaluation was done of the effect of replacement of wheat flour with the detoxified and decolorized *L. mutabilis* derivatives on the quality properties of three types of bread products (loaf, bun and sweet).**

AQ2 **RESULTS: Physicochemical and nutritional analyses coincided with previous reports. The *Lupinus* protein concentrate and isolate had lower phenolic compound and oligosaccharide •(3.6) concentrations than the untreated seeds (0.58). Amino acid composition was determined for wheat flour (WF), *L. mutabilis* defatted and detoxified flour (LF), *L. mutabilis* protein concentrate (LPC) and *L. mutabilis* protein isolate (LPI). The resulting values were used to calculate the replacement levels at which lysine content would be increased significantly in WF–lupin blends. Replacement levels were: LF (5%, 10%, 15% and 20%); LPC (2.5%, 5%, 7.5% and 10%); LPI (0.5%, 1%, 2%, 3% and 4%).**

**CONCLUSION: The detoxifying treatments employed decreased non-nutritional and toxic compounds present in original lupin seed. use of citric acid (1%) reduced yellow coloration in LF and LPC.**


**Keywords:** bread; *Lupinus mutabilis*; lupin flour; legumes

## INTRODUCTION

Lupin seeds are employed as a protein source for animal and human nutrition in various parts of the world, not only for their nutritional value (high in protein, lipids and dietary fibre), but also their adaptability to marginal soils and climates. Human consumption of lupins has increased in recent years. Lupin flour is added for its nutritive value (high protein efficiency ratio) and also to provide functional properties in bakery and pastry products. Seed has high protein (30–45g $kg^{-1}$) and oil (10–18 g $kg^{-1}$) content in some species.[1] Worldwide total cultivation of lupin is still limited and has never exceeded 7000 ha $y^{-1}$. However, the potential cultivation[2] area is estimated at around $10^6$ ha. About 90 species have been reported throughout Mexico. These wild lupins have not been exploited at a commercial level in countries such as Germany, Spain, Australia or South Africa.[3] The use of this crop as a source of food has been limited by the presence of toxic factors such as quinolizidine alkaloids (Qas); non-nutritional compounds such as the oligosaccharides (OGS) stachyose, raffinose and verbascose, which are not digested in the human intestine, and are flatulence-causing agents;[4,5] and phenolic compounds (PC) which interact with human salivary praline-rich protein to produce an astringent sensation and diminish protein digestibility through inhibition of enzymes.[6] It has also been suggested that the consumption of these compounds may also have beneficial effects on human health by reducing the risk of some diseases.[7] Nutritionally, *Lupinus mutabilis* significantly improves the amino acid balance, mainly

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by increasing lysine content, and is a good fibre source.[9–11] Addition of small quantities of *L. mutabilis* flour in replacement of cereal flour tends to improve baked product textural properties, flavour and often colour.[12] Some *L. mutabilis* species confer a yellow colour that is highly valued in foods such as pasta,[12] but constitutes a visual sensory disadvantage in others food products such as white bread. Addition of 4% white *L. mutabilis* flour to whole wheat flour results in slightly heavier bread due to the dough's increased water absorption capacity, but this property also increases shelf life.[12–16] Acceptability is very high for products with up to 10% added *L. mutabilis* flour,[15,12] and, in fact, *L. mutabilis* flour has been used at up to 50% replacement levels of wheat flour in biscuits, with good results.[15]

The benefits of this legume in baked goods can be brought to poorer populations in Mexico by adding *L. mutabilis* flour, and/or derivatives such as protein concentrate or protein isolate, to wheat flour used in a wide variety of commonly consumed, low-cost cereal-based foods such as leavened white loaf bread, bun bread and sweet bread. In an effort to increase the use of *L. mutabilis* in cereal-based foods in Mexico, the present study objective was to evaluate the decrease or elimination of non-nutritional compounds present in *L. mutabilis* derivatives, colour quality attributes and acceptability of Mexican-style loaf, white *bolillo*-type bread and sweet bread prepared with wheat flour enriched with different levels of *L. mutabilis* flour, protein concentrate or protein isolate.

## MATERIALS AND METHODS

### Raw material

*Lupinus mutabilis* var. multulopa seed (L) was acquired from the Institute of Technological Investigation, National Polytechnical School in Quito, Ecuador, and wheat (*Triticum aestivum*) var. •Pastor flour (WF) was a gift from the International Maize and Wheat Improvement Centre (CIMMYT) in Mexico.

### Chemical analyses

Protein (N × 6.25; method 955.04), lipids (method 920.39), crude fibre (method 962.09) and ash (method 923.03) were determined according to AOAC methods.[17]

### Detoxification and milling of *L. mutabilis* seeds

Detoxification of *L. mutabilis* seeds was done by first soaking in boiling water for 5 min, as recommended by Acuña and Ormaza,[18] followed by a continuous water wash for 15 h. The detoxified seeds were oven-dried at 60 °C for 4 h and milled using an electric coffee grinder until a coarse flour was produced.

### Decoloration of *L. mutabilis* flour with benzoyl peroxide and ascorbic acid

Benzoyl peroxide (100 ppm) and ascorbic acid (40 ppm) were added to *L. mutabilis* flour (LF) at levels permitted for their use as antioxidants in wheat flour.[19]

### Defatting and citric acid decoloration of *L. mutabilis* flour (LF)

LF was defatted by soaking in hexane (1:4, LF:solvent) for 8 h in a cold chamber under constant stirring. Once defatted, the flour was decoloured by soaking in water for 6 h, followed by addition of an aqueous 1.0% citric acid solution (1:4, LF:citric acid solution) every 30 min during a 90 min period.[20]

### *L. mutabilis* protein concentrate (LPC)

LPC was produced following the method of Fernández.[21] Briefly, one part detoxified, defatted LF was mixed with four parts 80% aqueous isopropyl alcohol for 30 min under constant agitation, the mixture allowed to rest for 3.5 h and the solubilized material decanted. The process was then repeated three times. A second protein concentration process was run using 60% aqueous isopropyl alcohol. All LPCs were freeze-dried, ground and sifted through 8xx mesh to produce a particle size similar to that of wheat flour.

### *L. mutabilis* protein isolate (LPI)

LPI was produced following the method of Onayemi and Lorenz.[22] Briefly, one part defatted LF was suspended in four parts water (w/v), and suspension pH adjusted to 9 with 0.1• mol $L^{-1}$ NaOH. The suspension was stirred for 30 min, centrifuged at 3000 × *g* for 15 min each time, and the precipitate extracted. This was repeated, producing a second supernatant, and decanted. The supernatants from both extraction steps were combined, placed in a centrifuge tube, pH adjusted to 4.6 with 0.1 mol $L^{-1}$ HCl in the new solution, the mixture stirred for 30 min and then centrifuged at 3000 × *g* for 10 min. The LPI (i.e., the resulting precipitate) was freeze-dried• the end test, ground and sifted through 8xx mesh to produce a particle size similar to that of wheat flour.

### Amino acid analyses

Amino acid composition of each studied sample – wheat flour, *L. mutabilis* flour, LPC and LPI protein – was determined by high-performance liquid chromatography (HPLC) according to Elkin and Wazynozuck.[23]

### Carbohydrate (CH) extraction and quantification

CH extraction from LF, LPC and LPI was done following the method of Muzquiz *et al.*[24] The different samples studied (•0.1G) were ground and then homogenized with aqueous ethanol solution (50% v/v, 5 mL) for 1 min at 4 °C and the supernatant was recovered. The procedure was repeated twice and the combined supernatants were concentrated under vacuum at 35 °C. The concentrated supernatant was dissolved in deionized water (1 mL) and passed through a Waters minicolumn (Waters C-18 at 500 mg

$mL^{-1}$) with a Supelco vacuum system (Waters, Milford, MA, USA).

Samples (20 µl) were analyzed using a Beckman HPLC chromatograph f156 with refraction index detector. A Waters Spherisorb 5-NH2 column (250 × 4.6 mm i.d.) was used with acetonitrile:water (65:35, v/v) as the mobile phase at a flow rate of 1 mL $min^{-1}$. Individual sugars were quantified by comparison with standards of sucrose, raffinose, stachyose and verbascose. Calibration curves were prepared for all these sugars and a linear response was obtained for the range of 0–5 mg $mL^{-1}$ with a determination coefficient ($r^2$) > 0.99.

### Tannin analyses

Tannin determination was done using the method of Singleton and Roos.[25]

### Colour analysis

Colour was determined with a Color Mate HDS color meter (Milton Roy Co., Ivyland, PA, USA), calibrated using a standard white tile. The test plastic bags, sealed with Ziploc™, measured 17 × 17 cm. A 500 g sample of flour was used. Three readings were taken per sample, and the results expressed as the average of CIELAB $L^*$, $a^*$ and $b^*$ uniform colour space, where $L^*$ indicates lightness, $a^*$ indicates hue on a green (−) to red (+) axis and $b^*$ indicates hue on a blue (−) to yellow (+) axis.[26]

### Wheat–*L. mutabilis* blends

Based on the amino acid profile results, and calculations of lysine content in the LF, LPC, and LPI, replacement percentages were determined for enrichment of WF. With the purpose of increasing lysine content in WF, the lupin flour and its derivatives were added at the following proportions: LF 5%, 10%, 15% and 20%; LPC 2.5%, 5%, 7.5% and 10%; LPI 0.5%, 1%, 2%, 3% and 4%.

### Preparation of white loaf bread

Dough was prepared as described in the standard 'Breadmaking Procedure' (AACC, Method 10-10B).[27] After mixing it was placed in a covered aluminium bowl (•Hobart), allowed to rest for 5 min and then manually kneaded; consistency was determined based on whether the dough stuck to the hands when separated. Floor time was 30 min, during which the dough was placed in a fermentation cabinet at 32 ± 2 °C and 75% ± 5% RH, and punched down once. The dough was then weighed (100 g), manually rounded and placed in individual metal bread moulds. Proofing was done for 30 min at 32 ± 2 °C, and 85% ± 5% RH, and baking was done in an electric rotary oven, for 24 min at 210 °C.

### Preparation of white *bolillo*-type bread

Bun bread, known as *bolillo* in Mexico, was prepared according to National Baking Industry Association methods.[28] Flour (1000 g), water, yeast, salt and fat were mixed together (Hobart), the dough divided into 50 g portions and shaped into the *bolillo* form. These were left to rise for 30 min at 30 °C, and then baked for 20 min at 200 °C.

### Preparation of Mexican-style sweet bread

Mexican-style sweet bread was prepared according to National Baking Industry Association methods.[28] Flour (1000 g), water, yeast, salt and fat were mixed together (Hobart), and the dough was divided into 50 g portions and shaped into different sweet bread forms. These were left to rise for 30 min at 30 °C, and then baked for 20 min at 200 °C.

### Bread firmness

Bread firmness was tested with a complete piece of bread in triplicate using a double compression test applied with a texture analyser (model TA.XT2, Texture Technologies Corp., Scarsdale, NY, USA). Samples were analysed 0 h and 24 h after baking, under the following equipment conditions: time 0 or 24 h; loading cell • 50 k; 25 mm lapped Perspex cylinder probe. Compression was increased from 0% to 20%, when force as a function of time was measured. The double compression test produces two curves. Firmness is the highest point on the first curve and is read directly on the graph. Three replicates were done per treatment to determine evaluation reproducibility.

### Bread volume

Bread volume was determined by the rapeseed displacement procedure[29] after cooling for 2 h.

### Sensory evaluation

An experienced baker scored crumb structure on a scale of 1 to 4 (i.e., poor, fair, good and very good, respectively) based on crumb cell size, shape and distribution. Taste acceptability was determined using 35 untrained judges, who scored product flavour on a 1 to 5 hedonic scale (i.e., 'like very much' to 'dislike very much'). Results were analysed with a one-way ANOVA.

### Statistical analyses

All results were statistically evaluated using analysis of variance (ANOVA) and correlation procedures.

## RESULTS AND DISCUSSION

### Chemical composition

The proximate composition analyses (Table 1) showed protein content to increase with defatting of the lupin flour from 34.0% in LF to 49.4% in LDF. This is higher than reported by Duque[30] (45.0%) and Acuña and Ormaza[18] (46.5%) for defatted *L. mutabilis* seeds, and the difference may be due to seed origin. The LPC protein •(70 ± 1.3) and fat contents (0.8 ± 4.5) were lower than values reported

**Table 1.** Chemical composition of wheat flour (WF), *L. mutabilis* flour (LF), *L. mutabilis* defatted flour (LDF), *L. mutabilis* protein concentrate (LPC) and *L. mutabilis* protein isolate (LPI)

| Component | WF (%) (N × 5.27) | LF (%) | LDF (%) | LPC (%) | LPI (%) |
|---|---|---|---|---|---|
| Moisture | 14.8 ± 0.2 | 7.1 ± 0.1 | 8.0 ± 0.4 | 6.4 ± 0.7 | 3.0 ± 0.3 |
| Protein (N × 6.25) for legumes | 10.0 ± 0.2 | 34.0 ± 2.0 | 49.4 ± 5.0 | 70 ± 1.3 | 93.5 ± 1.8 |
| Lipids | 1.3 ± 0.2 | 16.0 ± 1.4 | 0.8 ± 0.05 | 0.8 ± 0.05 | 1.0 ± 0.2 |
| Crude fibre | ND | 6.5 ± 1.3 | 3.2 ± 0.7 | ND | ND |
| Ash | 0.5 ± 0.1 | 2.8 ± 0.6 | 2.0 ± 0.5 | 2.0 ± 0.1 | 2.2 ± 0.3 |
| Carbohydrates (by difference) | 73.4 ± 0.2 | 33.6 ± 0.4 | 36.6 ± 1.2 | 20.8 ± 0.6 | 0.3 ± 0.3 |

Values are the mean ± SD of three replicates.

by D'Appolonia,[31] probably because of the different extraction methods.[32] Protein content in the LPI (93.5%) was similar to that reported for *L. albus* (95.7%).[33] The low fat content in the LDF (0.8%) confirmed that the extraction method eliminates a high proportion of fat (16%), and was equally efficient as that used by Duque.[30] Fat content in the LPI (1%) was slightly higher than that reported by •King[24] (0%) (1985). Fibre content in LF (3.2%) was lower than reported by Schoeneberger *et al*. (4.4%).[34] Both the LPC and LPI had no measurable fibre content (0%), although due to traces of fibre •a one way both had measurable ash content (2.0% and 2.2%, respectively). Moisture in the LF (12.0%) was higher than in the LPC (1.2%) and the LPI (2.1%).

## Amino acid composition

The essential amino acid profiles (Table 2) showed that lysine content was higher in the LF (7.3), LPC (6.8) and LPI (4.3) than in the WF (2.1). Lysine proportion decreased slightly with protein extraction, being lower in the LPC than in the LF, and lower in the LPI than in the LF and LPC. Lysine values reported in the literature[13] (Ballester) for other *Lupinus* variety seeds (*L. albus*, 4.2%; *L. luteus*, 3.8%) are lower than obtained here for *L. mutabilis*, perhaps because of residual fat content in the other varieties or differing environmental conditions[13] (Ballester). The lower lysine content in the LPI may be explained by the alkaline treatment (NaOH 0.1 mol $L^{-1}$, pH 9.3) employed for protein isolate extraction. This can lead to formation of lysinoalanine, a compound produced in some cereals when they are exposed to Na and K alkaline solutions.[36] The previous results and the calculations carried out with base in the lysine content in derivatives of *L. mutabilis* •showed higher lysine content, up to 18 g $kg^{-1}$ of protein.

**Table 2.** Amino acid composition of wheat flour (WF), lupin detoxified flour (LF), lupin protein concentrate (LPC) and lupin protein isolate (LPI) (g amino acid per 16 g N)

| | | | | | FAO/WHO[a] | | |
|---|---|---|---|---|---|---|---|
| Amino acids | WF | LF | LPC | LPI | 1 | 2 | 3 |
| Threonine | 1.7 | 3.2 | 3.6 | 2.9 | 4.3 | 3.4 | 0.9 |
| Tyrosine | 4.3 | 5.1 | 3.2 | 1.8 | – | 6.0 | – |
| Valine | 1.2 | 3.2 | 4.8 | 2.9 | 3.5 | 3.5 | 1.3 |
| Methionine + Cys[b] | ND | 3.0 | 0.8 | 0.7 | 4.2 | 2.5 | 1.7 |
| Isoleucine | 3.7 | 4.0 | 4.5 | 3.5 | 4.6 | 2.8 | 1.3 |
| Leucine | 6.8 | 5.7 | 8.1 | 7.0 | 9.3 | 6.6 | 1.9 |
| Phenylalanine | 4.7 | 3.2 | 4.5 | 4.2 | 7.2 | 6.3 | 1.9 |
| Lysine | 2.1 | 7.3 | 6.8 | 4.3 | 6.6 | 5.8 | 1.6 |

Values are the mean of three replicates.
[a] Recommendations from ref. 24: 1, children <2 years; 2, children 2–5 years; 3 adults.
[b] ND, not determined.

## Total carbohydrates and oligosaccharides

Total carbohydrates results (Table 3) showed the LDF to contain •9.3 g $kg^{-1}$ CH, consisting of sucrose and oligosaccharides: raffinose (2.29 g $kg^{-1}$); stachyose (4.12 g $kg^{-1}$); and verbascose (1.04 g $kg^{-1}$). The protein concentrate and protein isolate extraction protocols applied here reduced sucrose content slightly and substantially reduced oligosaccharides content, producing a total carbohydrate content 41% lower in the LPC and 61% lower in the LPI. Oligosaccharide content in the untreated LF was similar to that reported by Silva and Leite,[37] who indicated a reduction of 45% in the total CH of different *Lupinus* varieties by cooking for 60 min.

## Tannin compounds

The results obtained for tannin content in LF, LPC and LPI are presented in Table 4. The original content •in LF 2.5% and LFa lower than that obtained

**Table 3.** Carbohydrate content of lupin defatted flour (LDF), lupin protein concentrate (LPC) and lupin protein isolate (LPI) (g $kg^{-1}$)

| Treatment | Sucrose | Raffinose | Stachyose | Verbascose | Total CH reduction % |
|---|---|---|---|---|---|
| LDF | 1.82 ± 0.0 | 2.29 ± 0.85 | 4.12 ± 0.203 | 1.04 ± 0.3 | ND |
| LPC | 1.55 ± 0.09 | 1.12 ± 0.19 | 2.34 ± 0.029 | 0.45 ± 0.1 | 41.3 |
| LPI | 1.34 ± 0.17 | 0.82 ± 0.02 | 1.46 ± 0.024 | 0.00 | 61.1 |

Values are the mean ± SD of three replicates.

for soy bean flour. In LPC and LPI the content of these compounds •decreased until values were 38.8% and 59.6% less than that indicated for LF. Jimenez[20] reported a variety of *L. mutabilis* seed with a content twice as high as that found in *L. campestris*. These results are similar to those obtained by El-Adaway *et al*.[38] in *L. termis* •($0.32 \pm 0.04$) and *L. albus* ($0.42 \pm 0.05$). Lqari *et al*.[39] reported values of 0.1% for *L. angustifolius* flour and protein isolate.

## Decoloration

Decoloration of LF with benzoyl peroxide was largely ineffective since •*b* values were essentially the same as the blank (Table 5). Benzoyl peroxide reduces the yellow colour by degrading carotenoids, for example in wheat flour.[40] Lack of an effect in the blank and treated LF suggest that the yellow coloration in this lupin species is the result of phenolic compounds such as catechins,[40] which would explain why benzoyl peroxide had no bleaching effect.

**Table 4.** Tannin content of lupin flour without defatted (LFa), lupin defatted flour (LDF), lupin protein concentrate (LPC) and lupin protein isolate (LPI) (g $kg^{-1}$)

| Sample | Tannin content |
|---|---|
| LFa | $0.5837 \pm 0.14$ |
| DLF | $0.569 \pm 0.05$ |
| LPC | $0.3567 \pm 0.02$ |
| LPI | $0.2354 \pm 0.08$ |
| Soy bean flour | $0.5188 \pm 0.11$[a] |

[a] Source: •Jiménez.[33]
Values are the mean ± SD of triplicate determinations.

**Table 5.** Effect of benzoyl peroxide (100 ppm) and ascorbic acid (40 ppm) treatments on decoloration of lupin flour (LF)

| Sample/days of treatment | 0 days | 8 days | 15 days |
|---|---|---|---|
| LF | •$L = 98.83$<br>$b = 20.17$ | $L = 103.93$<br>$b = 21.23$ | $L = 103.93$<br>$b = 21.40$ |
| LF[a] | $L = 104.38$<br>$b = 21.28$ | $L = 103.93$<br>$b = 21.26$ | $L = 101.33$<br>$b = 21.02$ |

[a] Treated with 100 ppm benzoyl peroxide and 40 ppm ascorbic acid.

In response, a second treatment was applied utilizing an aqueous 1% citric acid solution as an antioxidant,[20] with increased soaking time followed by 12 washings (30 min per washing). This treatment partially decoloured after 6 h of continuous washing and very effectively decoloured after 8 h, producing a decrease in yellow colour (*b*) in both LF and LPC (Fig. 1). These results may be due to the presence of catechins in the tannins at pH values between 4.5 and 7.0.[40]

Extensive research has been done on the yellow colour given to final products by flours from soy,[41] some *L. mutabilis* varieties,[10,11] navy bean[41] and Great Northern bean.[29] Colour intensity increases in proportion to legume flour inclusion levels. This yellow colour is not necessarily disagreeable to trained panellists, and can even provide considerable appeal to products such as pasta and noodle dishes.[12] Nonetheless, yellow tonalities are not always desired, and so efforts have been made to reduce the yellow tones produced by *L. mutabilis* flours to the lowest possible levels. The procedure used here was effective in substantially lowering yellow tones in the studied *L. mutabilis* flours and derivatives, suggesting that it may have potential applications in developing new flour preparation technology.

## Bread product firmness

Overall, firmness at 0 h decreased in the loaf and *bolillo* bread products containing LF, LPC or LPI when compared to the respective bread products in the control (WF), but increased in the sweet bread products (Fig. 2). This variable increased in the loaf bread and sweet rolls containing LPC, but remained unchanged in *bolillo* bread with LPC. Addition of LPI increased firmness in the loaf bread and sweet bread, but decreased it in *bolillo* bread. At 24 h, the loaf bread and *bolillo* bread products with added LF tended to lose firmness or experience no change compared to their values at 0 h, whereas sweet bread products increased in firmness. The loaf, *bolillo* and sweet bread products containing LPC had similar firmness values at 24 h and 0 h, and those containing LPI had the same values.

The difference in firmness behaviour between bread products containing LF, LPC or LPI is probably

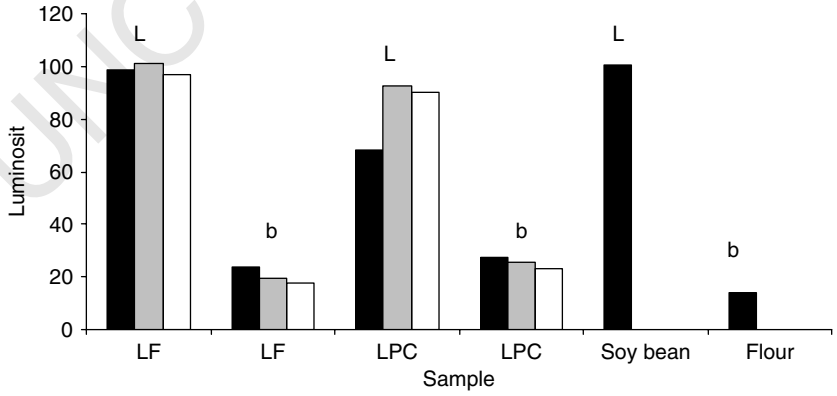


**Figure 1.** Effect of aqueous 1% citric acid solution on decoloration of lupin flour (LF) and lupin protein concentrate (LPC): 0 h (■); 6h (▩); and 8h (□).

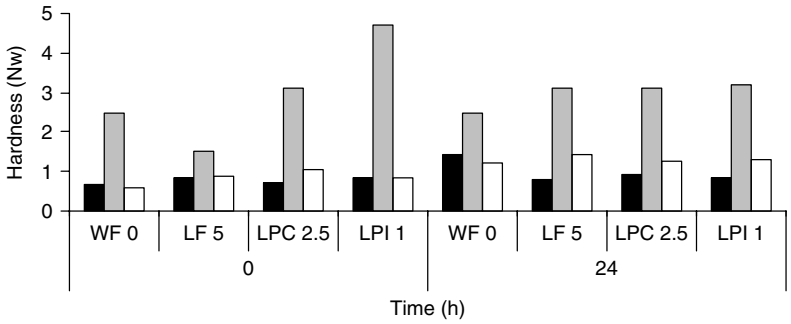


**Figure 2.** Firmness in loaf bread (■), *bolillo* bread (▩) and sweet bread (□) made with wheat flour (WF) and enriched with *L. mutabilis* flour (LF), protein concentrate (LPC) or protein isolate (LPI), at 0 and 24 h.

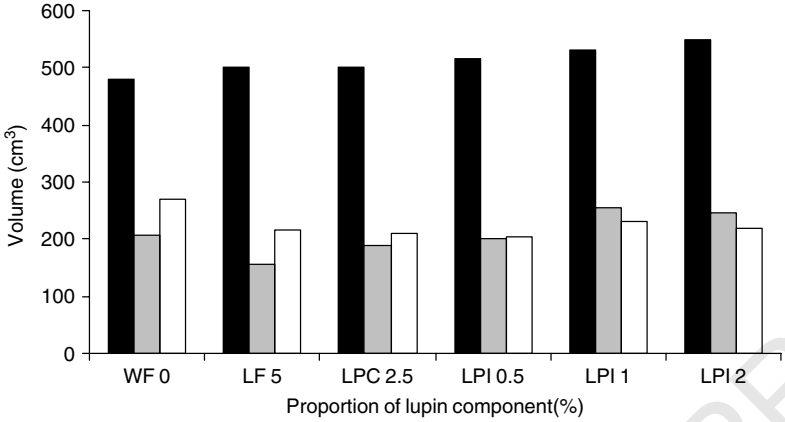


**Figure 3.** Volume ($cm^3$) in loaf bread (■), *bolillo* bread (▩) and sweet bread (□) made with wheat flour (WF) and enriched with *L. mutabilis* flour (LF), protein concentrate (LPC) or protein isolate (LPI).

the result of the higher protein and carbohydrate proportions in LF compared to LPC and LPI. Higher protein and carbohydrate contents increase firmness in bread products. These results are closely linked to results reported by Güemes *et al*.[42] They used microstructure studies of wheat flour doughs enriched with LF, LPC or LPI, and generated trough photomicrographs showing a progressive loss of interaction in the wheat gluten protein network with increasing lupin replacement levels. This compromises the bonds in the protein network since the wheat protein does not interact with the lupin protein and leads to empty spaces in the lupin-enriched bread products. Rheological analyses in the same study[42] indicated that the rheological properties of the doughs were modified by increasing levels of LF, LPC or LPI. At higher replacement levels, however, the lupin protein does interact with the gluten protein network, modifying the protein structure. This is reflected in rheological and texture properties, and may cause the higher firmness values in lupin-enriched products. Firmness can also be affected by other protein-containing ingredients such as eggs and milk, which, in conjunction with lupin additives, can increase product firmness. Depending on product end-use, this property can be considered either negative or positive, for instance by facilitating product transport. Campos and El-Dash[43] reported that in bread produced using an experimental baking test enrichment with 5% LF produced bread with quality characteristics similar to the control. Pollard *et al*.[44] reported that bread structure remains unaffected at up to 5% LF replacement levels.

## Bread product volume

Addition of LF and its derivatives had variable effects on bread product volume (Fig. 3). Compared to WF, addition of LF, LPC and LPI in loaf bread increased volume in all the lupin treatments.[45] In *bolillo* bread, volume decreased in the LF and LPC treatments, but increased at both LPI concentrations (1% and 2%). The sweet bread products fortified with LF, LPC or LPI were all slightly lower in volume than in the WF treatment.

The• increased volume in loaf bread enriched with lupin derivatives is probably due to the difference observed in the behaviour of the fortification on the volume of the loaf bread, and would explain the function of several factors: the different periods of fermentation applied in each case; and in laminate and rolled steps and the punched and bowled steps in white *bolillo*-type bread, a volume decrease was observed in addition to the different components of each formulation. On the other hand, the volume in the sweet bread diminished in all the proved cases. It is important to consider that given the viscoelastic properties of wheat protein, •it is thought that gluten net formation during fermentation would allow the

trapping of carbon dioxide. This would be modified by the presence of legume globular proteins, derivatives which do not interconnect with gluten proteins, giving as a consequence a smaller trapping capacity of the gas and therefore a smaller volume. A similar behaviour was obtained with microstructure.[29] The increased volume observed here coincides with results reported by Fleming and Sosulski[46] for loaf bread containing one of three different legumes. Other researchers have reported similar results. King[33] found that loaf bread containing 1% soy bean flour attained a higher volume, and Hoover[47] reported that bread fortified with 10% *L. mutabilis albus* flour had a higher volume than unfortified bread. •Dervas *et al*.[11] also observed a slight increase in the volume of bread containing *L. albus* flour, while Pollard *et al*.[44] reported that addition of 5% *L. albus* flour increased bread loaf height. Finally, other authors found that volume increased in bread containing up to 9% *L. mutabilis* flour.

### Sensory evaluation

The sensory test performed by a trained judge showed the most acceptable products to be those containing 5% LF, 2.5% LPC or 0.5% or 1% LPI (Table 6). Acceptance was based on the texture and colour of the lupin-enriched products.[8,9,10,48,49] In these products, crust colour was darker, crumb colour was more yellow and crumb texture showed evidence of thickened cells with addition of the flours. These coincide with other reported results.[49] Crumb quality in loaf bread with 2.5% LPC or 0.5% LPI was similar to that with 0% lupin additives (i.e., WF), which correlated with volume. This was not the case with the *bolillo* bread or sweet bread products, for which crumb quality was described as good to poor.

Sensory results from the panel of untrained judges showed the most acceptable products to be those fortified with 5% LF, 2.5% LPC or 0.5% or 1% LPI. Bread products made with unenriched WF were consistently evaluated as having good sensorial properties and were ranked higher than the lupin treatments. These results are similar to those reported by Clark and Johnson[49] in which the appearance, flavour and texture of foods fortified with *L. angustifolius* protein isolates were evaluated and accepted at a 95% confidence interval.

Colour values for the three bread types fortified with LF, LPC or LPI showed yellow coloration to be most intense in the sweet bread (Table 7). This property had very low values in the loaf bread enriched with 5% LF, 2.5% LPC or 0.5% or 1% LPI. The strong coloration in sweet bread products is not necessarily a negative sensory quality since this colour is normally pleasing to the consumer. Indeed, •Dervas *et al*.[11] reported that the yellow colours imparted by legume flours have considerable appeal and are thus potentially valuable additives in foods such as pasta and noodle dishes.

## CONCLUSIONS

Protein content was high in LF, LPC and LPI. Lysine concentration was 2.1% in WF, 7.3% in LF, 6.8% in LPC and 4.3% in LPI. These are appropriate amino acid levels for baked good additives. Modification of the decoloration procedure by increasing extraction

**Table 7.** Colour (•*b*) of loaf bread, *bolillo* bread and sweet bread made with wheat flour (WF), and enriched with *L. mutabilis* flour (LF), protein concentrate (LPC) or protein isolate (LPI)

| Sample | (*b*) value |
|---|---|
| WF | |
| 0 | 21.3 ± 0.4 |
| LF | |
| 5 | 22.6 ± 1.1 |
| 10 | 23.9 ± 0.3 |
| LPC | |
| 2.5 | 22.0 ± 1.4 |
| 5 | 26.0 ± 0.7 |
| LPI | |
| 0.5 | 21.0 ± 1.6 |
| 1 | 22.5 ± 0.8 |
| 2 | 22.7 ± 0.9 |

Values are the mean ± SD of three replicates.

**Table 6.** Sensory evaluation by a trained judge of loaf bread, *bolillo* bread and sweet bread (crumb colour and crumb texture) made with wheat flour (WF), and enriched with *L. mutabilis* flour (LF), protein concentrate (LPC) or protein isolate (LPI)

| | Loaf bread | | *Bolillo* bread | | Sweet bread | |
|---|---|---|---|---|---|---|
| Proportion of lupin component (%) | Colour | Texture | Colour | Texture | Colour | Texture |
| WF | | | | | | |
| 0 | Yellow | VG | Yellow | VG | Yellow | G |
| LF | | | | | | |
| 5 | Yellow | G | Yellow | P | Yellow | G |
| 10 | Very yellow | P | Very yellow | P | Yellow | G |
| LPC | | | | | | |
| 2.5 | Yellow | VG | Yellow | G | Yellow | G |
| 5 | Yellow | G | Yellow | P | Yellow | P |
| LPI | | | | | | |
| 0.5 | Yellow | VG | Yellow | G | Yellow | G |
| 1 | Yellow | G | Yellow | G | Yellow | G |

VG, very good; G, good; P, poor; R, regular.

time, applying continuous washes and the use of 1% citric acid effectively decreased yellow colour in LF, LPC and LPI. This provided favourable properties for loaf bread preparation and is thus a promising technological contribution to the production of certain lupin-enriched baked goods. Volume was optimum in the bread products enriched with 1% and 2% LPI. The bread products with firmness of texture from addition of 5% LF, 2.5% LPC or 0.5% or 1% LPI also manifested prolonged shelf life. Sensory evaluation of the lupin-enriched products by a trained judge based on colour and crumb texture indicated products containing 5% LF, 2.5% LPC or 0.5% LPI to be the most acceptable. Sensory evaluation of lupin-enriched loaf bread by untrained judges showed the products containing 5% LF, 2.5% LPC or 0.5% or 1% LPI to be the most acceptable. The most acceptable sensory evaluations for the sweet bread products was for products containing 5% or 10% LF, 2.5% or 5% LPC or 0.5%, 1% or 2% LPI. These evaluations coincide with the texture and volume results.

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## REFERENCES

1 Rodríguez ASL, Martínez AAL, Millan F and Dávila OG, Composition and functional properties of *Lupinus campestris* protein isolates. *Plant Foods Hum Nutr* **60**:99–107 (2005).

2 Imane TA and Al Faiz C, Lupin use and research in Mororro, in *Lupin: An Ancient Crop for the new Millennium: Proceedings of the 9th International Lupin Conference*, Klin/Müritz, Germany, pp. 20–24 (1999).

3 Aguilera JM and Trier A, The revival of lupin. *Food Technol* **8**:70–76 (1978).

4 Cumming JH and MacFarlane GT, The control and consequences of bacterial fermentation in human colon. *J Appl Bacteriol* **70**:443–459 (1991).

5 Piotrowicz-Cieslak AI, Michalcyk DJ and Gorecki RJ, Flatulence-producing oligosaccharides and galactosyl cyclitols in maturing Andan lupin (*Lupinus mutabilis*) seed, in *Recent Advances of Research in Antinutritional Factors in Legumes Seeds and Oilseeds*, ed. by Muzquiz M, Hill DD, Cuadrado C, Pedrosa MM and Burbano C. Wageningen Academic, Wageningen, Netherlands, pp. 73–76 (2004).

6 Bartolomé B, Estrella I and Hernández MT, Interaction of low molecular weight phenolics with protein (BSA). *Food Chem Toxicol* **65**:617–627 (2000).

7 Stravic B, Antimutagens and anticarcinogens in foods. *Food Chem Toxicol* **32**:79–90 (1994).

8 ••••••••••••••••

9 Aguilera MJ, Development of processes and uses of *L. mutabilis* for food. Doctoral thesis, Department of Chemical Engineering, Universidad Católica de Chile, Santiago de Chile (1993).

10 Mohamed AA and Rayas DP, Composition of *L. mutabilis albus*. *Cereal Chem* **72**:643–647 (1995).

11 Wittig de Penna E, Carreño P, Urrutia X, Lopez L and Ballester D, Sensory evaluation and acceptability of cookies enriched with sweet lupine flour (*L. mutabilis* albus cv. Multolupa). *J Food Sci* **52**:1434–1435 (1987).

12 Dervas G, Doxastakis G, Hadjisarva-Zinoviadi S and Triantafilakos N, Lupin flour addition to wheat flour doughs and effect on rheological properties. *Food Chem* **66**:67–73 (1999).

13 Ballester D, Zacarías I, García E and Yañez E, Baking studies and nutritional value of bread supplemented with full-fat sweet lupin flour (*L. mutabilis albus cv Multolupa*). *J Food Sci* **49**:14–18 (1984).

14 Campos EJ and El-Dash AA, Effect of Addition of full fat sweet lupine flour on quality of bread. *Cereal Chem* **55**:619–622 (1976).

15 Gammére CV, The production of micronised sweet white lupin flour for use as human food. *Adv Lupin Res* **1**:482–490 (1995).

16 Zacarías • and Yañez , Elaboración de Pan Fortificado con *L. mutabilis*. *J Food Sci* **45**:24–26 (1985).

17 AOAC, *Official Methods of Analysis* (15th edn). Association of Official Analytical Chemists, Washington, DC (1990).

18 Acuña • and Ormaza . Obtención de Harina, Concentrado y Aislado de Lupino (*L. mutabilis*). *J Int Sobre Prot Aliment* (2000).

19 Norma oficial mexicana NOM-147-SSA1-1996, bienes y servicios. Cereales y sus productos. Harinas de cereales, sémolas o semolinas. Alimentos a base de cereales, de semillas comestibles, harinas, sémolas o semolinas o sus mezclas. Productos de panificación. Disposiciones y especificaciones sanitarias y nutrimentales.

20 Jimenez MC, Obtención de un Analogo de Leche y de Yoghurt a Partir de la Semilla de *L. mutabilis campestris*. Master's thesis, Departamento de Graduados e Investigación en Alimentos del IPN México (1998).

21 Fernandez-Quintela A, Macarulla TM and Martínez AJ, Obtención y Caracterización de Concentrados de Proteína a Partir de Leguminosas. *Rev Esp Cienc Tecnol Aliment* **33**:285–297 (1993).

22 Onayemi O and Lorenz K. Soy concentrate and soy isolate in bread baking. *Bakers Digest* **12**:18–24 (1978).

23 Elkin RG and Wasynezuck AM, Amino acid analysis of feedstuff hydrolysates by precolumn derivatization with phenylisothiocyanate and reverse-phase high performance liquid chromatography. *Cereal Chem* **64**:226–229 (1987).

24 Muzquiz M, Cuadrado C, Ayet G, Cuadra C, Burbano C and Osagie A, Variation of alkaloids in seed of *Lupinus mutabilis*. *J Agric Food Chem* **42**:1447–1450 (1994).

25 Singleton VL and Roos JA, Colorimetry of total phenolics with phospomolybdic–phosphotungstic acid reagents. *Am J Enol Vitic* **16**:144–146 (1965).

26 Calvo A and Duran L, Propiedades Físicas II. Opticas y Color de Alimentos. *Temas Selectos en Tecnología de Alimentos*. Editorial CYTED-IPN, Mexico (1997).

27 AACC, *Approved Methods of the American Association of Cereal Chemistry*. AACC, St Paul, MN (1995).

28 CANAIMPA, *Manual de Operación*, Cámara Nacional de la Industria de la Panificación, Mexico (1998).

29 Güemes VN, Estudio del efecto de la adición de sustitutos de grasa a base de carbohidratos de donas fermentadas y no fermentadas. Master's thesis, IPN-ENCB, Mexico (1998).

30 Duque RL, Evaluación Química, Biológica y Teratogénica de *L. mutabilis*. Master's thesis, IPN-ENCB, Mexico (1995).

31 D'Appolonia LB, Use of untreated and roasted navy beans in bread baking. *Cereal Chem* **54**:53–56 (1978).

32 Liener IE, Implications of antinutritional components in soybean foods. *Crit Rev Food Sci Nutr* **34**:31–67 (1994).

33 King M, Obtention of isolate and concentrated of lupin for foods. *J Food Sci* **45**:321–324 (1985).

34 Schoeneberger H, Gross R, Cremer HD and Elmadfa I, Composition and protein quality of *L. mutabilis mutabilis*. *J Nutr* **112**:70–76 (1982).

35 FAO, *Protein Quality Evaluation*. Food and Agricultural Organization of the United Nations, Rome, p. 66 (1970).

36 Friedman M, Chemistry, biochemistry, nutrition and microbiology of lysinoalanine, lanthionine and histidinoalanine in food and other proteins. *J Agric Food Chem* **47**:1295–1329 (1999).
37 Silva HC and Leite BG, Effect of soaking and cooking on the oligosaccharides content of dry beans (*Phaseolus vulgaris* L.) *J Food Sci* **47**:924–925 (1982).
38 El-Adaway AI, Rahma HE, El-Bedawey AA and Gafar FA, Nutritional potential and functional properties of sweet and bitter lupin seed protein isolates. *Food Chem* **74**:445–462 (2001).
39 Lqari H, Vioque J, Pedroche J and Millan F, *Lupinus angustifolius* protein isolates: chemical composition, functional properties and protein characterization. *Food Chem* **76**:349–356 (2002).
40 Strack D, *Phenolic Metabolism*, *Plant Biochemistry*. Academic Press, London (1997).
41 Yousseff MA, Salem A and Hamid A, Supplementation of bread with soybean and chickpea flours. *J Food Technol* **11**:599–605 (1976).
42 Güemes VN, Arciniega RO and Dávila OG, Structural analysis of seed, flour, protein concentrates and isolates of *Lupinus mutabilis*, and their mixtures behavior with wheat flour. ••• •••••••••••••••••••••••• **37**:283–290 (2004).
43 Campos JE and El-Dash AA, Effect of addition of full fat sweet lupine flour on rheological properties of dough and baking quality of bread. *Cereal Chem* **55**:619–627 (1978).
44 Pollard NJ, Stoddard FL, Popineau Y, Wrigley WC and MacRitchie F, Lupin flours as additives: dough mixing, breadmaking, emulsifying, and foaming. *Cereal Chem* **79**:662–669.
45 Güemes VN, Características Reológicas, Sensoriales y Nutricionales de Masas y Productos de Panificación Fortificados con *Lupinus*. Doctoral thesis, ENCB-IPN, Mexico (2004).
46 Fleming ES and Sosulski WF, Breadmaking properties of four concentrated plant proteins. *Cereal Chem* **54**:1124–1140 (1972).
47 Hoover JW, Use of soy protein bakery. *J Am Oil Chem Soc* **51**:267–269 (1975).
48 Trejo CMG, Calderón G, García E and Farrera RR, Estudio del efecto de la adición de diferentes ingredientes sobre aqlgunas caracteristica de calidad en pan dulce tipo concha. *Rev Latinoam Inform Tecnol* **61**:16–42 (1995).
49 Clark R and Johnson S, Sensory acceptability of food with added lupin (*Lupinus angustifolius)* kernel fibre using pre-set criteria. *J Food Sci* **67**:356–362 (2003).

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