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Influence of storage time for the acceptability of bread formulated with lupine protein isolate and added brea gum

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ABSTRACT

The purpose of the research was to study the influence of storage time on the acceptability of bread made with lupine protein isolate and brea gum. Three bread formulations were studied: bread with wheat flour: lupine protein isolate (90:10) and brea gum, bread with wheat flour: lupine protein isolate (90:10) without brea gum, and a control bread (100% wheat flour). Texture Profile Analysis variables, moisture, dehydration rate, colour and acceptability were measure at fresh, 24, 48 and 72 h of storage. The crumbs made with flour mixture had more moisture at all storage times, and the addition of brea gum further increased this difference. After 24, 48 and 72 h of storage, the bread crumbs with lupine protein isolate (with and without brea gum) had a lower hardness ($^*P < 0.05$). In general, the addition of brea gum made breads more cohesive, gummy, springy and chewy ($^*P < 0.05$). All the crumbs tended to be less bright. At 48 h brea gum improved the acceptability ($^*P < 0.05$) and this was accentuated at 72 h of storage, where 80% of consumers had a positive acceptance because of the "*good crumb flavour*". The addition of this hydrocolloid increased the sensory shelf-life of the product.

1. Introduction

Lupine (*Lupinus mutabillis sweet*) is a leguminous plant, which has been used as food by people of the Andean highlands (Doxastakis, Zafiriadis, Irakli, & Tananahi, 2002). Lupine protein has a high nutritive value and the main interest relates to its high content of lysine (El-Adawy, Rahama, El-Bedawy, & Gafar, 2001).

Hydrocolloids are widely used to bake products for retarding staling and/or for improving the quality of fresh products (Hager & Aredent, 2013; Polaki, Xasapis, Fasseas, Yanniotis, & Mandala, 2010).

The brea gum (BG) is a hydrocolloid obtained as phloematic exudate of *Cercidium praecox,* specie of semi-arid regions of Argentina. The gum is collected manually by native people from superficial incisions made in the branches and trunks. BG is highly soluble in water (28.3% at 25 °C), and the solutions present acid character (pH = 4). This hydrocolloid contains residues of L-arabinose, D-xylose, D-glucuronic acid and 4-O-methyl-D-glucuronic acid (Cerezo, Stacey, & Webber, 1969), associated with small amounts of protein. BG has similar composition and structure to the arabic gum (De Pinto, Rodriguez, Martinez, & Rivas, 1993). Hence, BG could be a suitable candidate for incorporation as stabilizing, emulsifying and thickening additive.

Bread is essential in people's diet and one primary source of energy, as it is rich in carbohydrates, but is poor in quantity and quality of proteins (Bowles & Demiate, 2006). Moreover, it is a product characterized by a short shelf-life, resulting in the rapid onset of signs of staling, mainly related to the increase of the hardness of the crumb (Curti, Carini, Tribuzio, & Vittadini, 2014),

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which then affects its acceptability (Hough, Langohr, Gomez, & Curia, 2003). For these reasons, this paper aims at improving the quality of the protein in bread by incorporating lupine protein isolate, which has peculiar lysine content, and extends the lifetime of the product through the addition of a native hydrocolloid from Argentina.

Moreover, this study aims at sharing significant findings with the scientific community and the food industry. In addition, this investigation intends to contribute with the use of BG, which has recently been incorporated into the Argentine Food Code.

Finally, the objective of this research was to study the influence of storage time on the acceptability of bread, made with lupine protein isolate and BG.

2. Material and methods

2.1. Raw materials

Lupinus mutabillis Sweet seeds from Bolivia were used. Native BG was provided by indigenous communities from Chaco Salteño (Argentina). Commercial wheat flour (WF) (moisture: 10 g/100 g; protein: 11.8 g/100 g; ash: 0.71 g/100 g), compressed yeast, and other ingredients were purchased from local markets.

2.2. Lupine protein isolate (LI): obtaining, chemical composition and colour profile

Lupine seeds were crushed, using a household mill (Braun, Germany), and then defatted by soaking in petroleum ether (Cicarelli, analytical grade) for 20 h with four solvent changes. The defatted flour was air-dried at 25 °C and grounded to pass through a 0.173-mm ASTM sieve (80-mesh); it was used for preparing the protein isolate by alkaline water extraction/isoelectric precipitation, following the method proposed by El-Adawy et al. (2001).

Crude fat, protein, moisture and ash contents of LI were determined according to the AACC (2000) methods 30–10, 46–30, 44–15 and 08–01 respectively. Protein content was calculated with a 6.25 conversion factor. The carbohydrates were calculated by difference. Each analysis was performed for triplicate.

2.3. Purified brea gum

Grinding, dissolution, decantation, filtration and drying in an oven (at 30 °C), were the steps followed in the purification process. Since the BG has high solubility in water, the powder was solubilized in the water required for kneading to ensure a good distribution of the hydrocolloid throughout the dough (López, Pérez, Jiménez, & Cuevas, 2013).

2.4. Baking test and storage conditions

Table 1 shows the composition of the samples. Control bread was also elaborated.

Ingredients were mixed (10 min), kneaded and rolled in the commercial bread maker machine (ATMA easy cook). The dough was fermented (27°C-95 min), kneaded (25 min), and it was baked at 150 °C for 60 min. Finally, the breads were cooled to room temperature (120 min). For the study of ageing, the loaves were placed unpacked into a special camera and stored at 25 °C ± 2 °C with a 75–80% relative humidity for 24, 48 and 72 h. Three pieces of each type of bread were prepared and stored.

2.5. Physical parameters and chemical composition

Each loaf was characterized by, volume (V) (rapeseed displacement), specific volume (SV) (Dall'Asta et al., 2013), Specific volume index (SVI) (López et al., 2013) and Width/height ratio of the central slice (W/H) (Curic et al., 2008).

The analysis of the crumb structure was performed using the method and software proposed by Sciarini, Ribotta, León, and Pérez (2012). Calculations include: total area cells (%) (TAC), average size of the cells (mm) (ASC), and number of cells per unit area (n °C/cm^2). Three replicates for each sample were carried out.

Moisture, ash, crude fat and proteins, following the AOAC (1995) methods 925.09, 923.03, 922.06, 991.20 respectively, were analysed. The carbohydrate content was calculated by difference. Protein content was calculated using a 5.7 conversion factor. Three replicates for each sample were carried out.

2.6. Crumb staling evaluation

The breads staling was determined by the variation in the moisture (AOAC, 1995, method 925.09), dehydration rate (Davidou, Le Meste, Debever, & Bekaert, 1996), TPA parameters and colour, at different storage times.

The TPA was performed using a QTS 25 Texturometer (Brookfield, USA). A 2.5 cm thick slice was compressed with a 38.1 mm acrylic probe up to 40% deformation, at 120 mm/min speed and a relaxation period of 10 s between de first and second compression. An instrumental trigger of 5 g was applied. The hardness (g), cohesiveness, gumminess (g), springiness (mm) and chewiness were obtained. On average, six measurements per bread were made.

The colour of crumb was determined according to the CIELAB parameters (L^*, a^*, b^*) using a ColorTec, PCM colorimeter (Accuracy Microsensor Inc., Pittsford, USA), equipped with light source of D65 and an observation angle of 10°.

2.7. Overall acceptability and sensory shelf-life

Overall acceptability was measured in a total of 12 samples: WF, WF:LI, and WF:LI + BG at the four storage times. Regular consumers of bread, (203: 128 female, 75 male, aged between 18 and 40 years) evaluated the acceptability in a 9-point structured hedonic scale (9 = I like very much, and 1 = I dislike very much). Moreover, the following question was made: "*Would you consume this bread?*"

Table 1
Breads formulations (ingredients are expressed as percentage on a 100 flour/blend basis).

Bread	Ingredients				
	Wheat flour	Lupine protein isolate	Dried yeast	Salt	Brea gum
WF:LI	90	10	1.6	2	0
WF:LI + BG	90	10	1.6	2	0.5
WF	100	0	1.6	2	0

WF: wheat flour bread; WF:LI: wheat flour: lupine protein isolate bread; WF:LI + BG: wheat flour: lupine protein isolate with brea gum bread. The amount of water was calculated according to farinograph water absorption (data not shown).

Table 2
Chemical composition and colour profile of wheat flour and lupine protein isolate.

	Colour			Moisture	Ash	Protein g/100 g	Carbohydrate	Fat
	L*	a*	b*					
WF	94.2 ± 1.3	4.5 ± 0.4	6.8 ± 0.7	10.0 ± 0.8	0.7 ± 0.2	11.8 ± 0.2	70.2 ± 1.8	1.5 ± 0.2
LI	85.0 ± 3.5	7.0 ± 0.4	25.1 ± 1.3	3.2 ± 0.2	0.8 ± 0.2	92.3 ± 0.8	3.3 ± 0.4	**

Means ± standard deviations ($n = 3$). **no significant amount. WF: wheat flour; LI: lupine protein isolate.

while they also had to explain the reason for their decision. The sensory shelf-life was determined using the equations proposed by Hough et al. (2002) considering the acceptability.

3. Statistical analysis

One way ANOVA was carried out to assess the differences in physical and chemical characteristics in the fresh breads. ANOVA was performed to assess variables significantly different among samples using a model where storage time was taken at four levels, and type of bread at three levels. The interaction storage time × type of bread were considered fixed factors with factorial treatment arrangement (4×3, $k = 12$). When the interaction was significant, simple effects were analysed partitioning the ANOVA.

Multiple means comparisons were carried out by Tukey HSD test at $^*P < 0.05$. Means and standard deviations were also calculated.

The following Pearson's correlation coefficients were calculated: area fraction vs. average cells size and vs. specific volume; and hardness vs. moisture.

A principal component analysis (PCA) was applied to the mean variables of the TPA, acceptability, moisture, and colour of breads samples in order to integrate all instrumental and sensory data. A correlation matrix was used and the minimum eigen value was set at 1. For PCA, a multivariate regression analysis was used. Moreover, to search the natural groupings among the samples, the factors scores were subjected to Hierarchical Cluster Analysis to group similar patterns breads. The sample similarities were calculated on the basis of the squared Euclidean distance, and the Ward method was used to establish clusters.

To calculate the sensory shelf-life, a two-factor ANOVA (repetition and storage time) ($^*P < 0.05$) was performed on the consumer's overall acceptability data. Mean rating and Fisher's Last Statistical Difference for each term were calculated. A linear regression was carried out considering consumers' overall acceptability as dependent variable and storage time as an explanatory variable. Using this regression, the sensory shelf-life could also be determined as the time required for the acceptability scores of the product to reach a certain predetermined value or failure point (A_{min}).

Statistical analysis was performed using Infostat v.2012p®, registered by Universidad Nacional de Córdoba, Córdoba, Argentina.

4. Results and discussion

4.1. Characterization of LI

Table 2 shows the chemical composition of LI and WF. The values for LI are similar to the ones previously reported (El-Adawy et al., 2001 – protein: 91.2 g/100 g, ash: 1.35 g/100 g, lipids 0.21 g/100 g and moisture: 2.45 g/100 g). WF presents a chemical composition that agrees with the mean values expected for the product.

4.2. Physicochemical analysis of fresh loaves

Table 3 introduces data related to the physicochemical analysis of fresh bread. Breads made with LI showed a significant reduction ($^*P < 0.05$) of specific volume, and specific volume index. These results agree with those obtained by López (2014), Paraskevopoulou, Provatidou, Tsotsiou, & Kiosseoglu (2010), Doxastakis et al. (2002), El-Adawy (1997), who reported that incorporating lupine, soy, and sesame protein isolates in bread provides loaves with a lower specific volume. This decrease in volume could be due to the dilution of gluten and mechanical disruption of the gluten network structure by the lupine particles (Paraskevopoulou et al., 2010). The high resistance to extension exhibited by these systems may restrict expansion during fermentation and baking: too high resistance can induce a limited and slow expansion of the air cells during proofing. It can also be hypothesized that the lupine proteins suppress the amount of steam generated, as a result of their high water absorption capacity, leading thus to reduced loaf volume. The width/height ratio showed that breads with LI had less volume since slices were wider than higher. This result is probably related to the decreased

Table 3
ANOVA of physical variables and chemical composition of fresh bread samples.

	Variable	WF	WF:LI	WF:LI + BG
Physical	SV(cm^3/g)	2.26 ± 0.05b	2.13 ± 0.12a	2.10 ± 0.16a
	SVI (%)	100b	94a	93a
	W/H	1.19 ± 0.06a	1.73 ± 0.2b	1.71 ± 0.1b
	Average cell sice (mm)	3.84 ± 0.03b	2.82 ± 0.02a	2.75 ± 0.05a
	Area fraction (%)	30.55 ± 1.99b	28.90 ± 1.13a	27.93 ± 0.10a
	N° cells/cm^2	7.97 ± 0.42a	9.03 ± 0.11b	8.93 ± 0.20b
Chemical composition g/100 g	Moisture	44.6 ± 0.17a	46.58 ± 0.37b	47.55 ± 0.15b
	Protein	8.46 ± 1.08a	13.97 ± 1.14b	14.05 ± 1.06b
	Ash	0.51 ± 0.04a	0.54 ± 0.02a	0.55 ± 0.02a
	Fat	0.86 ± 0.02a	0.88 ± 0.01a	0.87 ± 0.04a
	Carbohydrates	45.30 ± 2.63b	37.87 ± 1.93a	36.38 ± 2.06a

Means ± standard deviations ($n = 3$). Values in the rows followed by the same letter are not significantly different ($^*P < 0.05$), according to Tukey's test. WF: wheat flour; LI: lupine protein isolate SV: specific volume; SVI: Specific Volume Index; W/H: width/height ratio; N° cells/cm^2: number of alveoli per unit area.

Table 4
Partitioned ANOVA by storage time factor of moisture, dehydration rate, other variables derived from the texture profile analysis (TPA) and colour profile.

Storage time	Bread	Moisture (g/100 g)	Dehyd. Rate (%)	Cohesiveness	Springiness (mm)	Gumminess (g)	Chewiness	L*	a*	b*
Fresh	WF	44.6 ± 0.17a	–	0.80 ± 0.10b	9.10 ± 0.03a	632.33 ± 12.50a	5753.98 ± 94.81a	74.83 ± 0.21b	5.10 ± 0.10b	15.10 ± 0.10a
	WF:LI	46.58 ± 0.37b	–	0.75 ± 0.05 ab	8.97 ± 0.07a	674.67 ± 5.51b	6051.50 ± 33.52b	58.24 ± 0.05a	−10.24 ± 0.25a	19.04 ± 0.12b
	WF:LI + BG	47.55 ± 0.15c	–	0.69 ± 9.11a	9.00 ± 0.11a	684.93 ± 5.00b	5716.17 ± 21.80a	58.20 ± 0.10a	−10.08 ± 0.03a	19.94 ± 0.12b
24 h	WF	44.01 ± 0.23a	1.23 ± 0.56a	0.63 ± 0.07a	8.50 ± 0.75a	599.33 ± 3.09a	4411.98 ± 26.75a	74.13 ± 0.06b	5.14 ± 0.05b	15,21 ± 0.04a
	WF:LI	45.95 ± 0.21b	1.24 ± 0.32a	0.68 ± 0.01b	8.89 ± 0.02b	721.00 ± 8.54c	6407.20 ± 64.94c	58.08 ± 0.08a	−10.25 ± 0.31a	19.05 ± 0.05b
	WF:LI + BG	46.95 ± 0.27c	1.33 ± 0.26a	0.62 ± 0.01b	8.92 ± 0.05b	663.97 ± 4.00b	5389.52 ± 62.92b	58.14 ± 0.05a	−10.39 ± 0.28a	19.98 ± 0.08c
48 h	WF	43.15 ± 0.25a	3.27 ± 0.35a	0.45 ± 0.05a	7.20 ± 0.20a	597.50 ± 2.50a	4321.67 ± 21.50a	73.63 ± 0.38b	5.08 ± 0.02b	16.60 ± 0.26a
	WF:LI	45.00 ± 0.33b	3.31 ± 0.32a	0.50 ± 0.10a	8.79 ± 0.09b	680.33 ± 10.50c	5975.80 ± 33.81c	57.08 ± 0.08a	−10.04 ± 0.05a	19.05 ± 0.05b
	WF:LI + BG	46.50 ± 0.24c	2.17 ± 0.95a	0.60 ± 0.01b	8.70 ± 0.07b	632.00 ± 6.00b	5498.26 ± 80.02b	57.09 ± 0.10a	−10.10 ± 0.04a	20.38 ± 0.54c
72 h	WF	42.00 ± 0.23a	5.86 ± 0.45b	0.30 ± 0.07a	6.50 ± 0.26a	567.00 ± 24.00a	3691.47 ± 30.75a	72.24 ± 0.07b	5.12 ± 0.05b	16.93 ± 0.06a
	WF:LI	44.20 ± 0.23b	5.00 ± 0.64b	0.52 ± 0.01b	8.68 ± 0.04b	696.77 ± 1.25c	6050.29 ± 35.29c	56.14 ± 0.14a	−10.05 ± 0.05a	19.57 ± 0.51b
	WF:LI + BG	46.00 ± 0.14c	3.22 ± 0.74a	0.57 ± 0.02b	8.61 ± 0.26b	672.53 ± 1.50b	5792.51 ± 273.87b	56.01 ± 0.12a	−10.10 ± 0.05a	20.60 ± 0.10c

Means ± standard deviations ($n = 3$). Values in the columns for each storage time, followed by the same letter are not significantly different (*P < 0.05), according to Tukey's test. WF: wheat flour bread; WF:LI: wheat flour: lupine protein isolate bread; WF:LI + BG: wheat flour: lupine protein isolate with brea gum bread.

elasticity of the dough that results from the addition of LI (data not shown). Thus, during the baking process, the loaves grow more in width than upward.

Differences (*P < 0.05) in the size and distribution of the cells were observed (Table 3). The crumbs with LI had a lower average size of cells and hence greater amount of these per unit area. This resulted in a lower area fraction, which is directly related to loaf volume. In this regard, it is noted that the area fraction had a high positive correlation with the average cell size (r = 0.97) and SV (r = 0.95). In addition, the area fraction of breads with LI, were lower than the control (Table 3). Large cells in wheat bread are due to gluten elasticity, but the lupine proteins are not as elastic as gluten (Paraskevopoulou et al., 2010), so they did not form a network which allows cell expansion. Consequently, the crumb appears more compact. Probably, the dough with LI could not entrap gas bubbles, resulting in a dense crumb structure (Chiavaro, Vittadini, Musci, Bianchi, & Curti, 2008; Dall'Asta et al., 2013). No difference was found (*P < 0.05) between WF:LI and WF:LI + BG breads, indicating that the presence of BG did not improve the volume in fresh bread, probably because it is not a gum with surface active properties such as HPMC -which forms an interfacial film at the boundaries of gas cells that possibly provides stability in the cells during expansion (Hager & Arendent, 2013)-. Contrary to alginates, BG not reduced the bread volume (Rosell, Rojas, & Benedito, 2001). The incorporation of BG did not affect the physical parameters, confirming that the volume and crumb structure variations were due to the effect of incorporation of LI in the formulation.

The LI increases the resistance to expansion of the dough during the fermentation process. Furthermore, the ability to absorb water of the LI could reduce the amount of steam generated during baking (Paraskevopoulou et al., 2010). Both factors result in a smaller size of the alveoli, a lower specific volume of bread and in a dense crumb structure (Chiavaro et al., 2008).

Table 3 also shows the chemical composition of breads. The samples made with LI had higher protein content (65%) than the control and improved the percentage of the daily reference value (% DRV): a portion of 50 g of WF:LI, offers 14% of the DRV.

Only the moisture content was modified by the addition of BG, since the degree of addition was the minimum necessary to fulfil the hydrocolloid function as a food additive (López et al., 2013).

4.3. Evaluation of the bread crumb staling

The ANOVA showed significant interactions in storage time × type of bread for all variables, except for a* [$F_{(6,24)} = 1.47$]:

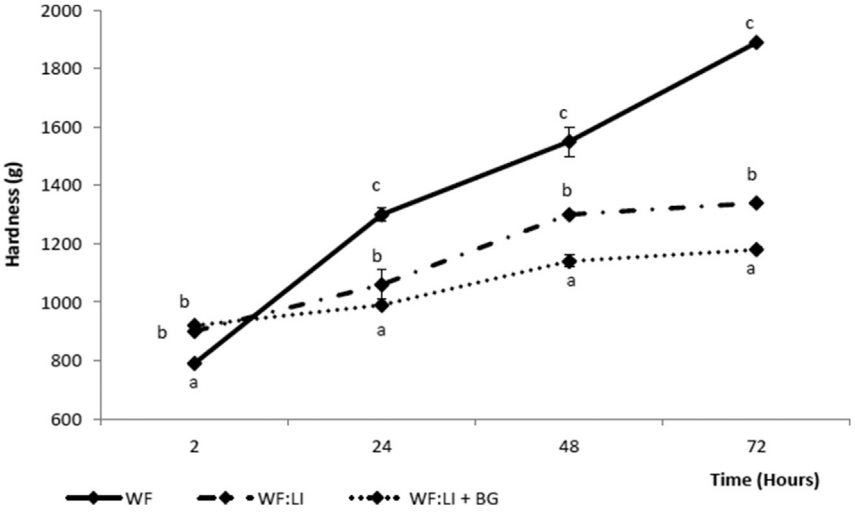

Fig. 1. Hardness variation of the bread crumbs during the storage time. Means ± standard deviations ($n = 3$). Points with the same letter for each storage times, are not significantly different (*P < 0.05), according to Tuckey's test. 2 h: fresh bread. WF:LI: bread made with mix of wheat flour and lupine protein isolate in 90:10 proportion; WF:LI + BG: bread made with mix of wheat flour and lupine protein isolate in 90:10, and with added of 0.5% w/w of brea gum.

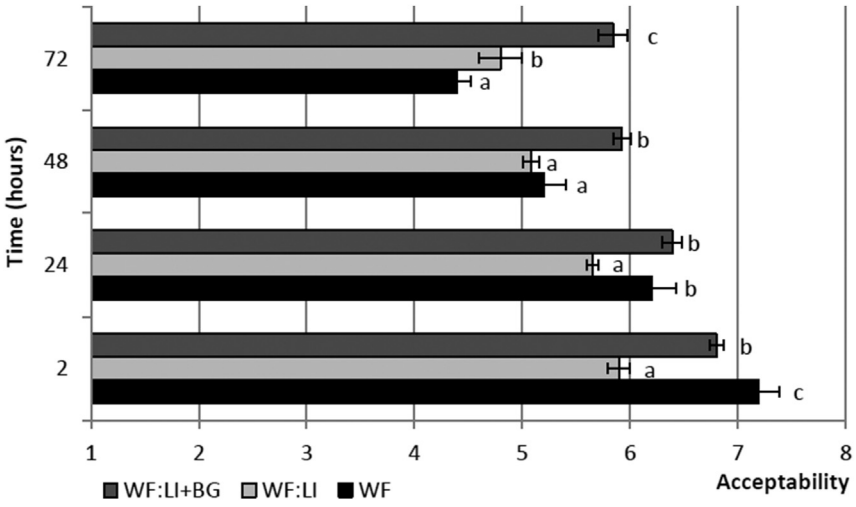

Fig. 2. Overall acceptability of high-protein breads with and without brea gum compared with the control bread at different storage times. Values in the bars followed by the same letter are not significantly different (*P < 0.05), according to Tuckey's test. 2 h: fresh bread. WF: wheat flour bread; WF:LI: flour: lupine protein isolate bread; WF:LI + BG: flour: lupine protein isolate with brea gum bread.

moisture [$F_{(6,24)} = 2.95$], dehydration rate [$F_{(4,18)} = 4.97$], cohesiveness [$F_{(6,24)} = 4.35$], springiness [$F_{(6,24)} = 61.72$], gumminess [$F_{(6,24)} = 124.38$], chewiness [$F_{(6,24)} = 103.23$], hardness [$F_{(6,24)} = 173.03$], L* [$F_{(6,24)} = 397.00$] and b* [$F_{(6,24)} = 17.20$]. Therefore, partitioned ANOVA for the cited variables was performed for each storage time, and these results are shown in Table 4.

Concerning the moisture of crumb samples, it can be observed that fresh control bread was less wet (*P < 0.05) than WF:LI and WF:LI + BG loaves. This coincided with the data reported by Paraskevopoulou et al. (2010), who found that the addition of 10% of LI to the bread, resulted in a greater water holding capacity, provided by the proteins. Moreover, fresh crumbs of WF:LI + BG bread were moister than those without the hydrocolloid, which is consistent with other works (Bárcenas & Rosell, 2007; Ghodke-Shalini & Laxmi, 2007; Guarda, Rosell, Benedito, & Galotto, 2004). At 24, 48 and 72 h (Table 4), the crumbs made with LI were significantly (*P < 0.05) moister, this is probably due to the fact that lupine proteins retain more water than gluten as it was suggested by Paraskevopoulou et al. (2010), because the dough formulation needed a greatest amount of water (measured with a farinograph test – data not shown). The addition of BG further increased this difference (*P < 0.05). Water plays a fundamental role in bread staling: a macroscopic migration of water occurs from crumb to crust, and it is redistributed in bread components (Korus, Witczak, Ziobro, & Juszczak, 2015). Gums are hydrophilic components which increase water retention in food systems and help to reduce the loss of the moisture during the storage (Guarda et al., 2004). As others hydrocolloids, the BG retains water in its structure; thus, moisture transfer and loss is limited (Davidou et al., 1996).

For the dehydration rate, significant differences were only detected after 72 h for WF:LI + BG. In this case, a partitioned ANOVA by type of bread indicated that the control bread and the bread with LI experimented a greater dehydration rate than the sample with BG (*P < 0.05) (data not shown).

Fig. 1 shows the evolution of hardness in control breads crumbs. As can be seen, fresh crumbs with LI were more hard due to a strengthening of the gluten network given by lupine proteins (Paraskevopoulou et al., 2010) and to the effect of the more compact crumbs (lower average size of alveoli; see Table 3). The added of hydrocolloid had no effect on fresh bread. After 24, 48, and 72 h, the breads crumbs with LI had lower hardness, because the moisture content was higher than the control (Table 4) (*P < 0.05). Also, the addition of the hydrocolloid emphasizes this effect. This was expected, because as in other studies (Guarda et al., 2004; Hager & Arendent, 2013), a high negative correlation (*P < 0.05) between moisture and crumb hardness was observed in WF:LI breads ($r^2 = -0.91$) and WF:LI + BG breads ($r^2 = -0.85$). The influence of

Table 5
Main reasons of consumption or not-consumption (>80%) of fresh and stored bread samples.

Storage time	Samples		
	WF	WF:LI	WF:LI + BG
	Main reasons (%)		
Fresh	Taste and/or Fluffiness (98%)	Soft and/or Moist (90%)	Wet and/or Tender and/or Good taste (97%)
24	Good flavour (85%)	Soft crumb (85%)	Tender and/or Tasty (90%)
48	Dry and/or Dark (90%)	Dry (80%)	Tender and/or Tasty (83%)
72	Very hard and/or Stale flavour (95%)	Hard and/or Stale flavour and/or Dry (90%)	Good flavour (80%)

WF: wheat flour bread; WF:LI: wheat flour: lupine protein isolate bread; WF:LI + BG: wheat flour: lupine protein isolate with brea gum bread.

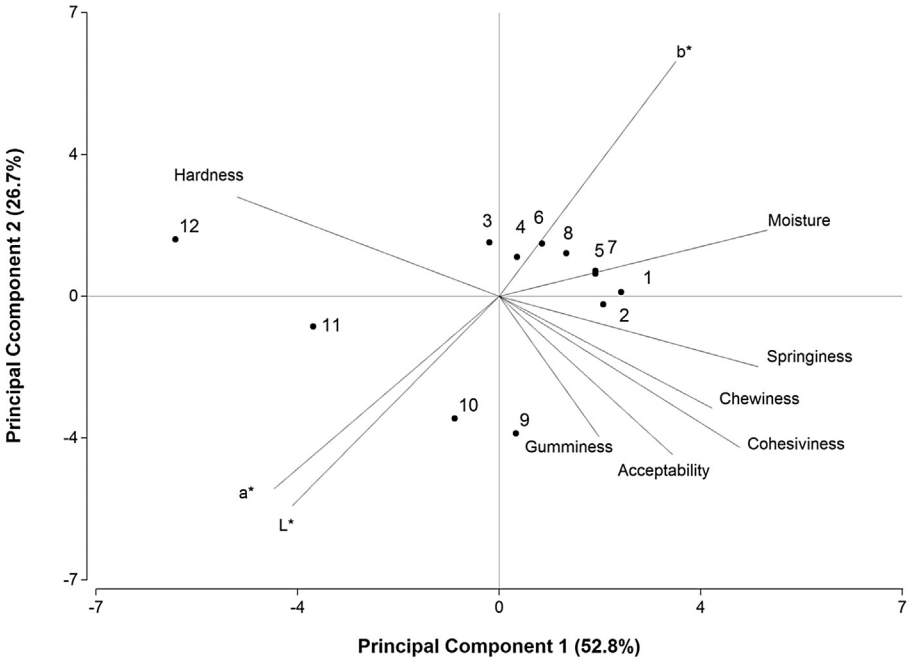

Fig. 3. Principal component analysis of instrumental and acceptability data for 12 bread samples. Samples 1, 2, 3 and 4: breads made with lupine protein isolate, at 2 (fresh), 24, 48 and 72 h of storage respectively. Samples 5, 6, 7 and 8: breads made with lupine protein isolate and brea gum, at 2 (fresh), 24, 48 and 72 h of storage respectively. Samples 9, 10, 11 and 12: control breads at 2 (fresh), 24, 48 and 72 h of storage respectively. PC: principal component.

the hydrocolloid on the hardness of bread crumb might result from the changes that occur in the amorphous part of crumb. Perhaps, hydrocolloid could inhibit starch–gluten interactions or the development of macromolecular entanglement (Davidou et al., 1996).

The cohesiveness (Table 4), indicates that all breads crumb, except the control at 72 h, had values greater than 0.5, hence they were more elastic than viscous. In general, as they age, the crumb structure lost their ability to remain joined (Chiavaro et al., 2008), and this behaviour was more marked in control bread crumbs. The gradual loss of moisture was possibly responsible for this result. With the addition of BG, the crumbs were more cohesive ($^*P < 0.05$) than the control one, after 24 h. This least variation suggests that the quantity of gum added, led to a more integrated matrix, in agreement with Zandonadi, Bothelo, and Araújo (2009) who worked adding psyllium in bread making.

In reference to the springiness (Table 4), the results show that this variable decreased with storage in the control bread, which is related to the lower cohesiveness and the highest dehydration rate. The bread crumbs made with LI, showed significant springiness ($^*P < 0.05$). Notably, no differences were observed in the values for this variable derived from the use of hydrocolloid, suggesting that

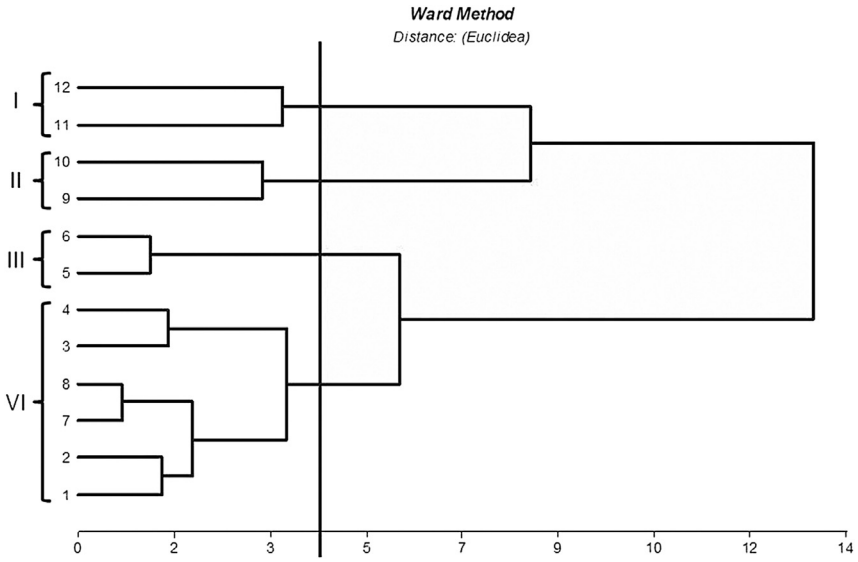

Fig. 4. Dendogram of cluster analysis. Samples 1, 2, 3 and 4: breads made with lupine protein isolate, at 2 (fresh), 24, 48 and 72 h of storage respectively. Samples 5, 6, 7 and 8: breads made with lupine protein isolate and brea gum, at 2 (fresh), 24, 48 and 72 h of storage respectively. Samples 9, 10, 11 and 12: control breads at 2 (fresh), 24, 48 and 72 h of storage respectively. PC: principal component.

Table 6
Sensory shelf life of bread samples calculated by FSMD criterion.

Bread	F	A_{min}	Equation of the line	R^2	End of shelf life (hours)
WF	7.20	5.70	$y = -0.94x + 8.1$	0.997	40.3
WF:LI	5.90	4.80	$y = -0.38x + 6.3$	0.989	72.0
WF:LI + BG	6.80	5.70	$y = -0.332x + 7.075$	0.938	99.4

F: Overall acceptability for the fresh breads; A_{min}: minimal acceptability. FSMD: first significant minimum difference. R^2: determination coefficient for acceptability vs. storage time. WF: wheat flour bread; WF:LI: wheat flour: lupine protein isolate bread; WF:LI + BG: wheat flour: lupine protein isolate with brea gum bread.

the addition of protein isolate had a predominant effect.

Regarding to the gumminess (Table 4), the WF crumbs were less gummy at all storage times, and this effect may be due by the influence of water absorption capacity and variations in chemical compositions (Bhol & Don Bosco, 2014). After 24 h, samples with BG were less gummy (*P < 0.05) than those made with LI. This was due to the higher hardness given by the lower moisture content in samples with LI.

Concerning chewiness (Table 4), the control samples were less chewy during storage due to the higher dehydration rate, causing drier and less cohesive crumbs. After 24 h, and from this interval onwards, the breads with LI had the most chewiness (*P < 0.05) and samples with BG showed intermediate values. It was observed that the samples with gum required less chewing work, which is justified because it is less gummy and hard.

Regarding colour (Table 4), the control breads were brightest (*P < 0.05), and the crumbs made with flour mixes are darker, depending of the nature of the flours (Villarino, Jayasena, Coorey, Chakrabarti-Bel, & Johnson, 2015). In general, all the crumbs tended to be less bright with time, but this was more evident in the control. This may be a minor characteristic, but may affect the acceptability of the stored product. The variable a* did not change with the storage. Evidently, breads with lupine had higher tendency towards a green colour and the addition of gum showed no effect (*P < 0.05). Respect to b*, crumbs with LI were more yellow (*P < 0.05). Samples with hydrocolloid had higher b* values (*P < 0.05) starting from 24 h, and this could be due to a slight golden colour of the BG.

4.4. Overall acceptability

With respect to the hedonic response (Fig. 2), the ANOVA showed significant interactions storage time × type of bread [$F_{(6,24)} = 28.23$]. As expected, the acceptability of bread decreased during the storage. In general, the acceptability for control breads decrease with time (*P < 0.05), while for the loaves with hydrocolloid achieved better scores.

Between fresh samples, the control bread was the most accepted (*P < 0.05), but at 24 h, the samples with BG were as accepted as the control. At 48 h breads with BG had more acceptability (*P < 0.05) and this was accentuated at 72 h of storage. In addition, Table 5 summarizes the main reasons for consumption. It should be noted that the "*dark*" characteristic appears as a reason of not consumption, and this can be related to the result of colour profile. The fresh bread with BG would be consumed by most participants (Table 5), but a 3% of consumers detected higher moisture in this sample *("very wet"* and even *"raw"),* and this is an interesting result, since this was a factor of not consumption.

4.5. Relation between instrumental data and acceptability: PCA and cluster analysis

The PCA is shown in Fig. 3. The first two principal components accounted for 79.5% of the total variance among the samples. This value was considered high, as other authors found values between 61% and 70% when introducing sensory data in the PCA analysis (Hobbs, Ashouri, George, Lovegrove, & Vodovotz, 2014; Jesen, Oestdal, Skibsted, & Thybo, 2011). With respect to the y-axis to the right, breads with LI and BG and the fresh control were associated with more acceptability, related to the moisture, cohesiveness, gumminess, chewiness and springiness. Conversely, the lower acceptability was associated with the hardness variable relating to the control samples with more than 48 h of storage. It can be also noted that all breads with BG were located in the first quadrant and related to higher moisture.

The results obtained in the cluster analysis are shown as a dendogram (Fig. 4). Considering a distance of 4.0, four groups can be identified: cluster I: samples of control bread at 48 and 72 h of storage (samples associated to hardness, opposed to acceptability); cluster II: fresh and at 24 h control breads (related to L*, a*, most TPA variables and acceptability); cluster III: breads whit BG fresh and at 24 h and cluster IV: all samples with LI and the samples with BG at 48 and 72 h (both related to moisture and b*).

In summary, the acceptability of samples was more related to moisture and TPA variables' than the components of colour, and specifically opposed to hardness.

4.6. Sensory shelf life of the breads (SSL)

Acceptability is affected by a variety of sensory, cultural and emotional factors. This variability makes consumers the best sensory judges. As the consumer decides the quality of the final product, it is possible to determine the time during which a food is acceptable for consumption by consumers, and after which, the product "fails". The ageing is a bread failure (Giménez et al., 2007).

Table 6 summarizes the data related to the SSL of the breads, using the criteria of First significant minimum difference (FSMD). The SSL of the bread is increased by the addition of the LI and better increased by the hydrocolloid. The control bread ends its shelf-life when the acceptability drops to 5.70 (This is based on the equations proposed by Hough et al., 2002) and this value will be reached at 1.68 days (Table 6), when the bread is neither liked nor disliked to the consumers. The SSL of the WF:LI bread expires when the acceptability reach the 4.80 points (I dislike nor dislike − I dislike slightly), and this punctuation was reached at 72 h of storage. Noticeably, the SSL of bread with BG would reach 99.40 h, according to the equation of the line, which is time that exceeds this study (Table 6). These data are consistent with the reasons for consumption (80% of consumers still would consume WF:LI + BG bread at 72 h). An important finding of this research is that, the SSL of bread is achieved by extending the action of this novel hydrocolloid.

5. Conclusion

All breads with BG were more accepted than their peers from the 24 h of storage: the main reason for this result is the high moisture, the lowest hardness and/or dry sensation on these crumbs. This study also confirms that the addition of BG increases the sensory shelf life of bread.

The principal component analysis revealed that the acceptability of samples was more related to moisture and TPA variables than components of colour, and opposed to hardness.

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