



Gluten-free baked muffins developed with *Prosopis alba* flour

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ABSTRACT

Baked flour products are basic in people's diet; the problem arises when celiac people tend to follow this kind of diet. In this work we exploit the benefit of American carob, *Prosopis alba* flour (PAF) in developing gluten-free muffins jointly with corn flour (CF) and rice flour (RF). All muffins obtained presented very good physical and sensory characteristics. The muffin with the highest amount of PAF presented low chewiness and was the most adhesive. Crumb structure of muffins with PAF presented alveoli of more irregular form although they occupied a greater proportion of area, leading to softer crumbs. Sample with high content of PAF presented the greatest score for sensory overall acceptability and the lowest for chewiness; also the intensive brown color was also well accepted by consumers. Nutritional analysis of the muffins indicate that those products with the highest content of PAF (66.6%) presented higher amount of proteins, ash and dietary fiber and lower of lipids. Also, these muffins presented a high amount of polyphenols and the highest antioxidant activity. Sensory and nutritional analysis showed that PAF contributed in a positive form to muffin's quality, suggesting that this flour would be a suitable ingredient as mimetic of chocolate-based breads.

1. Introduction

Cereal-based products mainly baked ones, constitute basic food for diet of people due to its sensorial and nutritional quality. Nevertheless, celiac people cannot consume this kind of products. Celiac disease is an auto-immunological unhealthy condition triggered by the ingestion of wheat, barley, rye and oat proteins, commonly named gluten proteins. These molecules induce an inflammatory response in the small intestine, resulting in atrophy of intestinal villi, hyperplasia of Lieberkühn crypts and infiltration of T lymphocytes (Green & Jabri, 2006). Elimination of these toxic prolamins from diet lead to a clinical and histological improvement of patients (Alaedini & Green, 2005). Celiac people consume a variety of food without gluten such as vegetables, non-processed meat and other processed products like bread, pasta and biscuits formulated with starches and gluten-free flours acquired in specialized markets (Lee, Ng, Zivin, & Green, 2007). The most difficult replacement for celiac people is bread and pastry products. Nevertheless, a great variety of gluten-free products are offered in the market and have been designed for celiac people to have an alternative to their common diet.

Gluten-free bread present several technological deficiencies such as low volume, insufficient color, shredded crumbs, among others (Matos Segura & Rosell, 2011). A great variety of gluten-free bread was

designed to bring celiac people a wide range of alternative products to their diet; they are usually formulated with corn (*Zea mays*) or rice (*Oryza sativa*) flours also combined with other starches (potato, tapioca) (Matos Segura & Rosell, 2011). Rice flour is one of the most convenient ingredient for these kind of products due to its hypoallergenic properties, the absence of color and taste. The low content of prolamins in rice that are not allergenic for celiac people, conducts to the necessity of using gums or hydrocolloids, emulsifiers, enzymes or lactic products (whey proteins) to obtain dough with the adequate viscoelastic properties (Demirkesen, Mert, Sumnu, & Sahin, 2010).

Baked products like muffins are very popular because they are consumed at breakfast or as a snack. They are sweet highly caloric products, very appreciated by consumers due to their good taste and smooth texture. The common mix for preparing traditional muffins is formulated with wheat flour, vegetable oil, eggs, sugar and water and/or milk. In the aqueous phase proteins, sugar and minerals are dissolved, while lipids are usually emulsified and starch granules and insoluble proteins are dispersed in the batter emulsion. The heating process during baking produce starch gelatinization and protein denaturation, leading to gelation and the formation of a baked matrix with air bubbles inserted in. Muffins usually present high volume with a porous structure that confers a spongy texture. This structure is achieved if dioxide carbon gas bubbles are retained in the continuous

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phase, contributing to the increase of bread volume after baking. One of the great challenges for obtaining high quality gluten-free products is to find adequate ingredients. Besides, some technological aspects should be considered if muffins will be developed without gluten. In this case, it is necessary to use a structuring agent to mimic gluten, such as egg proteins (Deora, Deswal, & Mishra, 2015) or hydrocolloids (Anton & Artfield, 2008). Tsatsaragkou et al. (2014, 2017) studied rheological properties of carob gluten-free dough and quality attributes of gluten-free breads prepared with a carob/rice blend with 15% of carob flour from *Ceratonia siliqua* L.; nevertheless no research was previously performed on gluten-free breads made with American carob (*Prosopis spp.*). Therefore, the objective of this work was to exploit the benefits of *Prosopis alba* flour (PAF) in development of gluten-free muffins. Firstly this flour has no toxic prolamins. Secondly, it contains more than fifty percent of soluble sugars, mainly sucrose (Sciammaro, Ferrero, & Puppo, 2016) that can replace the use of refined white sugar. The objective was to obtain sweet muffins using American carob flour in a mix with rice and corn flour assigned to celiac people and evaluate physicochemical, nutritional and sensory properties of these baked products.

2. Materials and methods

2.1. Experimental and mixture design

Muffins were developed with the following ingredients (g/100 g crude product): flour blend (25g), sucrose (6 g, Ledesma, Bs.As., Argentina), skimmed milk (35 g, La Serenísima, Bs.As., Argentina), fresh liquid egg (6 g), vegetable margarine trans fatty acid free (6 g, Dánica dorada, Avex S.A., Bs.As., Argentina), yeast powder (1 g, Levex, Bs.As., Argentina). Improvers used were 0.2 g sodium hydrogen carbonate (Royal, Bs.As., Argentina), 0.2 g salt (Celusal, Bs.As., Argentina), 0.4 g hydroxypropylmethylcellulose HPMC (Dow, USA) and 0.2 g sodium stearoil lactilate SSL (Danisco, Sao Paulo, Brasil). Flours used for the blend were rice (Ganofi, Santa Fe, Argentina) (RF), corn (CF) and *Prosopis alba* (PAF) obtained by grinding pods and sieving (500 µm) according to Sciammaro et al. (2016) methodology. Combinations of the three flours selected RF, CF and PAF were obtained with the DesignExpert 7.0 (StatEase INC) software. It was achieved to a simplex lattice design of second order that was obtained leading to ten experimental points with a duplicate of the central point (Table 1).

2.2. Preparation of muffins

Powder ingredients (flour, sodium bicarbonate, salt, HPMC and SSL) were mixed and sieved (1000 µm) for uniform size particle and then mixed with sugar and margarine. Eggs were whisked with a Philips

Table 1

Experimental design of flour blends used for muffins. Codified values: C1 (corn flour), C2 (rice flour), C3 (*Prosopis alba* flour). Decodified values (F1, F2, F3): values expressed in mass, g flour/100 g blend.

Blend	CF		RF		PAF	
	C1	F1 ^a	C2	F2 ^a	C3	F3 ^a
1	1	25	0	0	0	0
2	0	0	1	25	0	0
3	0	0	0	0	1	25
4	0.5	12.5	0.5	12.5	0	0
5	0.5	12.5	0	0	0.5	12.5
6	0	0	0.5	12.5	0.5	12.5
7	0.666	16.66	0.166	4.16	0.166	4.16
8	0.166	4.16	0.666	16.66	0.166	4.16
9	0.166	4.16	0.166	4.16	0.666	16.66
10 a	0.333	8.33	0.333	8.33	0.333	8.33
10 b	0.333	8.33	0.333	8.33	0.333	8.33

^a g/100 g blend.

(Philips Cucina HR 1566) mixer and therefore the necessary amount for the mixture was included. Yeast was incorporated to milk for activation process. The batter preparation was performed in the Philip mixer as follows: margarine was whipped with sugar 2 min at 711 rpm up to achieving a cream. Milk first and after that the liquid egg was incorporated to this cream, mixing each ingredient 1 min at 711 rpm. Sieved solids were included and mixed 2 min at 858 rpm. Finally, a semi-liquid system was obtained and 35 g incorporated to silicon conical circular moulds (volume: 90 cm³). Samples were fermented in a chamber at 30 °C during 40 min and then baked 25 min at 180 ± 10 °C in an electric oven. Muffins were let for cooling and stored in nylon bags during 24 h at 20 °C before using for analysis.

2.3. Determination of fermentation time

Optimum fermentation time was determined using the muffin mixture belonging to the central point, containing equal quantities of each flour (RF, CF, PAF). The batter was fermented at different times: 10, 20, 30, 40, 50 and 60 min and then baked during 25 min at 180 °C ± 10 °C. Fermentation time was selected by visual observation of the products (Fig. 1). The fermentation time selected was 40 min, because lower times produced muffins of too low volume, while higher times (50 and 60 min) led to disintegrated products.

2.4. Evaluation of physicochemical properties of muffins

2.4.1. Specific volume and color

The bread volume was determined in five bread samples of each mixture, by seed displacement in a loaf volume meter. Specific volume (cm³/g) was calculated as the ratio between bread volume and the weight of each piece. Height of each piece of muffin was also measured and it was performed in ten pieces.

Color of muffins was measured in crust of ten pieces for each mixture using a tristimulus colorimeter (Minolta CR400, Osaka, Japón) measuring L*, a* and b* color parameters of the CIELab color space. Luminosity L* present values from black to white (L* = 0, Black and L* = 100, White); a greater L* value indicates a lighter sample color. Values of a* parameter varies from red (positive values) to green (negative values) and b* parameter varies from yellow (positive values) to blue (negative values).

2.4.2. Preparation of crumb samples

Muffins were transversally cut at the middle height, using the inferior portion. Samples of crumb were obtained from the central part using a punch (diameter: 3.08 cm, height: 1.4 cm). These samples were utilized for moisture and texture analysis.

2.4.3. Crumb moisture

Slices of crumb were heated at 105 °C up to constant weight. Constant weight was considered when pieces achieved a mass difference between two consecutive weight measurements less than three percent. Determination was performed by triplicate.

2.4.4. Crumb texture

Texture of crumbs was determined in a texturometer (TA.XT2i Stable Micro Systems, UK) equipped with a 25-kg load cell. Pieces of crumb (n = 10) were subjected to a double compression at 40% of deformation using a cylindrical probe of 75 mm diameter (SMSP/75) with a speed of 0.5 mm/s. Parameters such as elasticity (dimensionless), cohesiveness (dimensionless), adhesiveness (N.s) and chewiness (N) were calculated from the force vs. time curves according to Bigne, Puppo, and Ferrero (2016).

2.4.5. Crumb alveoli properties

Crumb characteristics of muffins were assessed using a digital image analysis system. Slices of 5 cm of diameter were used for the analysis.

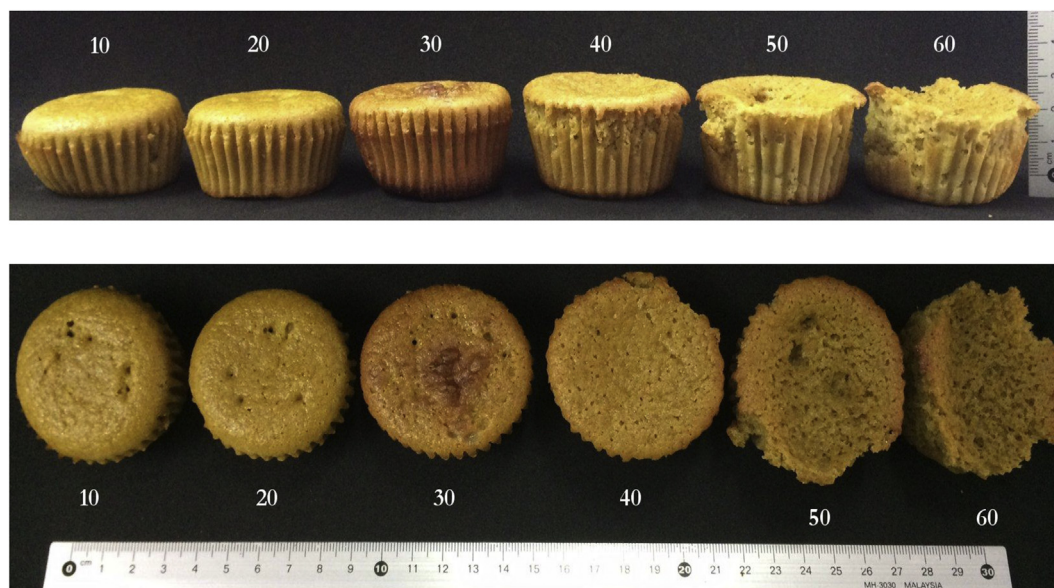


Fig. 1. Muffins elaborated at different fermentation times. (a) side view, (b) top view.

Images were acquired at 138 dpcm with a HP Scanjet 4070 Photosmart scanner (Hewlett-Packard, Palo Alto, CA, USA), processed using ImageJ software (V. 1.46r, National Institute of Health, USA) and converted to 8 bits grey-level image. The segmentation of the image (binary image conversion) was performed using the software for automatic selection of the threshold value. The binary image has only two grey levels: 0 for empty areas (black, air alveoli) and 255 for the walls of the alveoli (white bread crumbs). Object category (air cells) was assigned to those image zones that had a grey intensity between 0 and the threshold value. Crumb characteristics of muffins studied were: numbers of alveoli per area or density (N/cm^2), mean cell area (mm^2), circularity (1 maximum circularity, 0 without circularity), total area occupied by alveoli (%).

2.4.6. Sensory analysis of muffins

Sensory analysis was performed with a panel of 76 non-trained persons using hedonic scale of 9 points, assigning value “1” to the option “dislike” and “9” to the option “like”. Attributes evaluated were overall acceptability, color, aroma, taste and chewiness. Data of attributes were analyzed by ANOVA with $p < 0.05$ and mean values were compared with Fisher test.

2.5. Nutritional analysis of muffins

2.5.1. Proximal composition

Proximal composition of the muffins selected for the sensory analysis (Mixtures 5, 6, 9 and 10) was performed according to AOAC methods (AOAC, 1998). Moisture of samples was determined by triplicate after drying under vacuum at 50 mBar and 70 °C up to constant weight. Protein content was determined according to Kjeldahl method; the factor utilized for conversion nitrogen to protein was 6.25. Lipid content was determined using Soxhlet method; samples were dried 2 h at 100 °C and extracted during 2 h with petroleum ether (35–60 °C fraction). Total dietary fiber was determined by enzymatic hydrolysis (Megazyme kit, K-TDRF, Megazyme, Wicklow, Ireland) according to the AOAC method. Total soluble polyphenols were determined according to Sciammaro et al. (2016).

2.5.2. Sugar characterization

Soluble reducing sugars were determined by Somogyi-Nelson method using a calibration curve with glucose as standard according to the method developed for *Prosopis spp.* flours by González Galán,

Correa, Patto de Abreu, and Piccolo Barcelos (2008).

Soluble sugars also were identified and quantified by HPLC method described by Eliasson (2006). Lipids were extracted from 1 g of each sample with hexane at 40 °C, stirred 1 hr at 650 rpm and centrifuged 10 min at 2655 g. Supernatant was discarded and the pellet was treated with MiliQ water (13 mL) and solutions of potassium ferrocyanide (15% w/v, 1 mL) and zinc acetate (30% w/v, 1 mL). Dispersions were stirred at 70 °C during 30 min, and after reaching room temperature, acetonitrile (10 mL) was added and were centrifuged 10 min at 2655 g. Supernatants were filtered with a filter of 0.45 μm of pore diameter. Extracts were analyzed using an HPLC Waters 1525 (Millipore Corp., Milford, MA, USA). A Hypersil Gold Amino 250 column (i. d.: 4.6 mm, large: 25 cm) with particle size of 5 μm maintained at 35 °C was utilized. A system of acetonitrile:water 80:20 was used as mobile phase at a flow rate of 1.2 mL/min. A detection system that used measurements of refraction index was used. Calibration curve was performed with standards of glucose, fructose and sucrose (20 mg/mL, Sigma Corp.) and areas of peaks were analyzed by PeakFit v4.12 software (Systat Software, California, USA).

2.5.3. Polyphenol content

Polyphenols were extracted using an aqueous solution of acetone (50%) with a solvent:sample ratio of 3:1. Dispersions were stirred in a termomixer (Eppendorf Termomixer Comfort, Eppendorf, Hamburg, Germany) at 650 rpm during 40 min at 4 °C and then were centrifuged in a micro-centrifuge at 2655 g during 10 min at 20 °C. Polyphenol content and antioxidant activity were determined in the supernatants obtained. Total polyphenols content was determined according to Sciammaro et al. (2016) using the Folin-Ciocalteu method and values were expressed as gallic acid equivalents.

2.5.4. Antioxidant activity

Antioxidant activity was determined using the method of the [2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] radical (ABTS \bullet +) (Sciammaro et al., 2016). A stock solution 7 mM of ABTS \bullet + ammoniac salt containing 2.45 mM of potassium persulfate was prepared and stored overnight in the dark at 20 °C. This solution of ABTS \bullet + was diluted with ethanol up to obtaining an absorbance value of 0.700 ± 0.03 at 734 nm. One mL of the ABTS \bullet + ethanolic solution was added to 10 μL of the acetone sample extract; this new solution was mixed during 20 min and the absorbance was measured at 734 nm. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was

used as standard and results were expressed as Trolox Equivalent Antioxidant Capacity (TEAC) in μmol Trolox equivalents/100 g of dried sample.

All assays were performed by triplicate.

2.6. Statistical analysis

Differences were analyzed comparing average data values according to the Fisher test at $p < 0.05$ of significance level using the software Origin 9.0 Pro. Response Surface and the corresponding models for each variable were obtained through the DesignExpert 7.0.0 software. Only models that presented a significant fit ($p < 0.05$) were considered and for that purpose a lack of fit test was used; the model was valid with a lack of fit with $p > 0.05$.

3. Results and discussion

3.1. Experimental design

Data obtained from the DesignExpert 7.0.0 program are shown in Table 1, where the different quantities of each flour (rice flour RF, corn flour CF, *Prosopis alba* flour PAF) were informed for each point of the model. The total content of flour mix was 25%, while the other ingredients remained constant (75%), respect to 100 g of total mixture.

Fermentation time was selected utilizing one of the mixtures of the design (mixture 3). Fig. 1 shows that the adequate fermentation time was 40 min. For lower times (< 40 min) leavened was incomplete, while for higher times (50 and 60 min) a collapse of bread structure was observed.

3.2. Aspect and color of muffins

Fig. 2 shows all muffins obtained with the design of Table 1. It can be observed that algarrobo flour confers a brownish color to product, probably due to a great development of Maillard reactions during baking. These reactions favored by this process, require high temperatures and dehydration, and they are produced between reducing sugars and amino acids. According to Sciammaro et al. (2016), this *Prosopis alba* flour present a certain amount of reducing sugars (glucose and fructose) and high content of sucrose that would experiment partial hydrolysis and therefore might contribute to browning reaction.

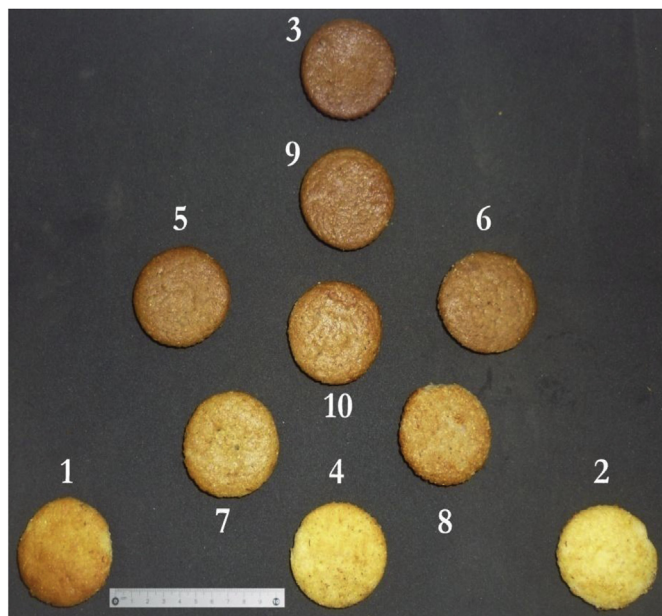


Fig. 2. Muffins of the different points of the experimental design.

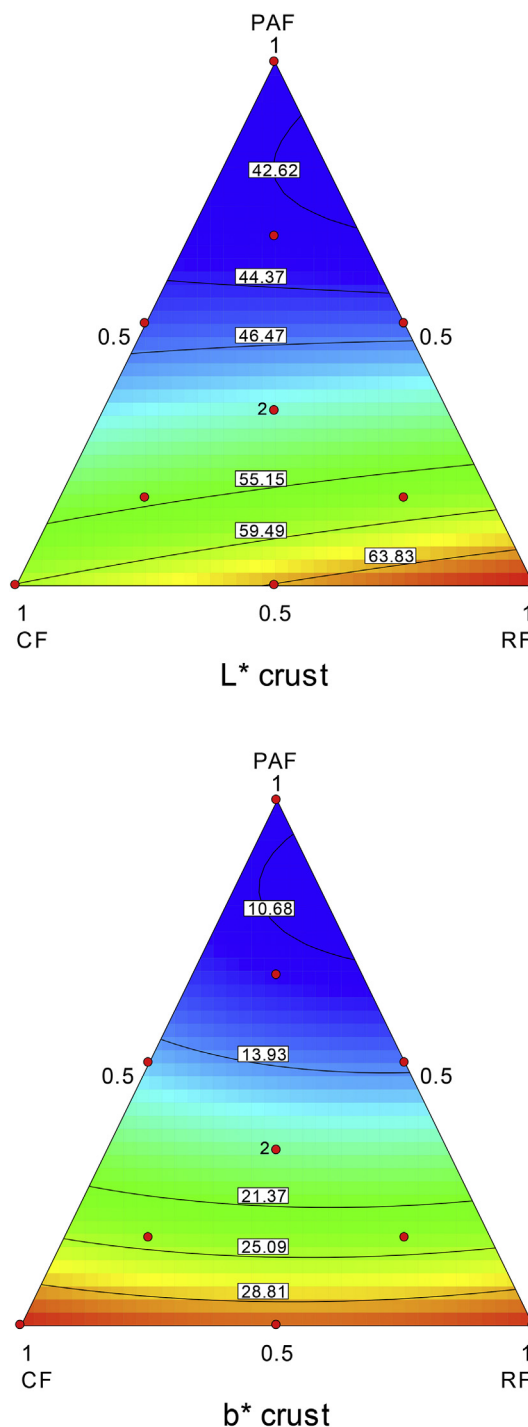


Fig. 3. Surface contour graph for color variables of muffin's crust: (a) luminosity- L^* (red: major L^* , blue: lower L^*); (b) blue to yellow coordinate- b^* (red: major b^* , blue: lower b^*). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Experimental determination of crust color of muffins are shown in Fig. 3, through the contour graph of surface response of luminosity (L^*) and blue to yellow coordinate (b^*) parameters. Parameter a^* (green to red coordinate) values did not fit the experimental model. The greatest values of L^* were obtained for samples prepared only with corn (M1, 59.01 ± 3.45) and rice (M2, 68.88 ± 1.85) flours, and also in the 1:1 blend of those flours (M4, 67.30 ± 1.80); being the most lightness samples those containing RF without significant differences in L^* parameter. On the other hand, the lowest luminous sample was that

formulated with PAF (M3, 42.75 ± 1.75), followed by sample M9 with a significant different L^* value of 44.75 ± 1.54 . Samples M5 and M6 presented values of L^* non significant different (46.83 ± 1.24 and 48.34 ± 0.80 , respectively); the same behavior was observed for samples M7 and M8 (54.42 ± 1.79 and 53.26 ± 1.66 , respectively).

Values of parameter L^* adjusted to the following quadratic model ($p_{\text{lack fit}}: 0.05282$, $p_{\text{model}}: 0.0048$, $r^2: 0.93$):

$$L^* = 59.64 \text{ CF} + 68.16 \text{ RF} + 43.94 \text{ PAF} + 0.54842 \text{ CF.RF} - 25.19 \text{ CF.PAF} - 41.61085 \text{ RF.PAF}$$

Parameter b^* presented values between 10.31 (M3) to 33.81 (M4). The highest values of b^* denote a higher tendency to yellow color on samples without PAF (M1, M2, M4), presenting no significant differences between parameters. Contour surfaces of b^* (Fig. 3) show that muffins with CF and RF presented the highest values of this parameter, and a decrease with the increase of PAF.

Values of parameter b^* adjusted to the following quadratic model ($p_{\text{lack fit}}: 0.0717$, $p_{\text{model}}: 0.0032$, $r^2: 0.94$):

$$b^* = 32.52 \text{ CF} + 32.41 \text{ RF} + 11.31 \text{ PAF} - 5.94 \text{ CF.RF} - 28.35 \text{ CF.PAF} - 33.30 \text{ RF.PAF}$$

Bigne et al. (2016) obtained similar results on breads prepared with mixes of wheat flour (WF) and *Prosopis alba* flour (PAF). In these breads, WF was replaced by PAF at levels of 15, 25 and 35%. These authors reported a similar trend with a decrease in L^* and b^* parameter with the increase of PAF.

3.3. Specific volume and moisture of muffins

Values of specific volume (cm^3/g) of muffins were in a range of 2.05 ± 0.02 (M1) to 2.52 ± 0.02 (M10b), and a value of 2.37 ± 0.02 was obtained for M10a. The highest values of specific volume were obtained for the mixture prepared with equal parts of the three flours; while the muffin containing only PAF presented an intermediate value of this parameter (M3: 2.30 ± 0.03) with non-significant difference with sample M9 (2.17 ± 0.02).

Moisture values are dependent on cooking time which in this case was the same for all mixtures. Although significant differences between samples were obtained, moisture value was around 49 g/100 g product with an interval of 48.28 ± 0.40 (M7)– 49.55 ± 0.78 (M8). These results suggest that moisture is not a variable that might influence muffin's quality.

3.4. Crumb alveoli

Different parameters of crumb alveoli are shown in Fig. 4. The greatest area occupied by alveoli was for those muffins containing higher amounts PAF (M3, M5, M6, M9). They also presented a higher individual surface (1.94 – 2.54 mm^2), comparing to those elaborated with RF (M2), CF (M1) or the mix of both flours (M4) (1.16 – 1.75 mm^2). Those alveoli were less regular as it can be deduced from the highest perimeter values (Fig. 4). Muffins enriched in PAF also presented alveolar densities (number of alveoli/ cm^2) significantly higher, being M3 (only PAF) the sample with the highest value. On the other hand, M9 and M5 did not present significant differences in alveoli density, while M6 that contains PAF + RF presented a density significantly lower than M5 (PAF + CF). These results suggest that corn flour (CF) better contributes to generate crumbs with high amount of alveoli than rice flour (RF). Contour surface (Fig. 4) for alveolus surface shows that this parameter is higher with higher amounts of PAF, but also shows that mixture tolerate the incorporation of certain proportion of CF without altering these values (red to yellow zone). Equations for the different models belonging to surface response are the following:

$$\text{Area} = 9.68 \text{ CF} + 11.15 \text{ RF} + 22.33 \text{ PAF} - 6.75 \text{ CF.RF} + 31.88$$

$$\text{CF.PAF} + 24.17 \text{ RF.PAF} - 115.13 \text{ CF.RF.PAF} \text{ (Model: special cubic, } p_{\text{lack fit}}: 0.862, p_{\text{model}}: 0.005, r^2: 0.96).$$

$$\text{Surface} = 1.67 \text{ CF} + 1.12 \text{ RF} + 1.93 \text{ PAF} + 1.39 \text{ CF.RF} + 1.98 \text{ CF.PAF} + 3.88 \text{ RF.PAF} - 19.98 \text{ CF.RF.PAF} \text{ (Model: special cubic, } p_{\text{lack fit}}: 0.972, p_{\text{model}}: 0.0449, r^2: 0.90).$$

$$\text{Perimeter} = 4.74 \text{ CR} + 4.35 \text{ RF} + 7.07 \text{ PAF} + 1.311 \text{ CR.RF} + 2.98 \text{ CR.PAF} + 4.60 \text{ RF.PAF} - 32.78 \text{ CF.RF.PAF} \text{ (Model: special cubic, } p_{\text{lack fit}}: 0.97, p_{\text{model}}: 0.01, r^2: 0.95).$$

$$\text{Alveolar density} = 5.84 \text{ CR} + 9.78 \text{ RF} + 11.66 \text{ PAF} - 11.08 \text{ CF.RF} + 7.13 \text{ CR.PAF} - 6.67 \text{ RF.PAF} + 43.78 \text{ CR.RF.PAF} \text{ (Model: special cubic, } p_{\text{lack fit}}: 0.571, p_{\text{model}}: 0.0016, r^2: 0.98).$$

Bigne et al. (2016) obtained for breads with 15% of replacement of wheat flour with PAF, approximately equivalent to mixture M9 of this work, an alveolus density of 18 alveoli/ cm^2 ; while for breads with 25% of PAF, equal in level of American carob flour to M3, they obtained 22 alveoli/ cm^2 . Even though the quantity of alveoli obtained by those authors was really higher, probably due to the effect of gluten proteins on crumb structure development, the same tendency was observed; high amounts of PAF lead to a great amount of alveoli per area. The same behavior in very different matrices, one with gluten and the other one without it, would be related to the effect of American carob flour on fermentation process. *Prosopis alba* flour has a high content of sucrose, higher than 50% (Sciammaro et al., 2016) that probably influence fermentation process, increasing the rate of reproduction of yeasts. Bigne et al. (2016) also found less alveolar surface with high content of PAF; in our work, PAF mixed with 50% of corn (M5) or rice (M6) flours presented the highest individual alveolus size (Fig. 4). Results suggest that PAF favors alveoli production, but these alveoli that are forming in a more rigid network due to the presence of components like fiber dilate with difficulty leading to alveoli of more irregular form with high perimeter. Corn starch of CF would act as structuring agent due to gelatinization during baking and gelation after cooling, consolidating crumb structure when American carob flour is combined with other flours that contain starch, particularly corn flour that improves alveolar morphology and this behavior could be related to a better crumb quality.

3.5. Texture of muffins

Values of texture parameters are shown in Fig. 5. Muffin prepared with CF presented the hardest crumb, while that with PAF was the softest crumb (data not shown). In wheat breads with PAF opposite results were obtained by Bigne et al. (2016), these authors informed that hardness increase with the inclusion of PAF in all substitution levels. A parameter related to hardness that better describe texture during biting is chewiness. This parameter decreased with the increase in PAF, a more soft product was obtained with only PAF (M3) or more than 50% of this flour (M9), as it can be also observed in the contour graph (Fig. 5, blue zone).

Elasticity is defined as the height that crumb recovers during the period between the second and the first cycle of compression and is calculated as the ratio distances from the origin to the maximum of the second peak to the first peak. The more elastic crumbs were those that contained RF (M2), CF (M1) and the mix of both flours (M4). Also mixtures with low quantities of PAF (M7, M8) and M10 (central point) presented high values of this parameter. Incorporation of higher amounts of PAF (M5, M6) significantly decreased elasticity. On the other hand, the softener crumb was observed for PAF muffins (M3). Results suggest that low amounts of CF and RF are sufficient for maintaining crumb elasticity; this parameter significantly decrease when they are absent from mixture. This behavior can be observed as the blue-light blue zone for PAF in the contour graph (Fig. 5). Texture properties of crumbs with high content of PAF can be related to the high

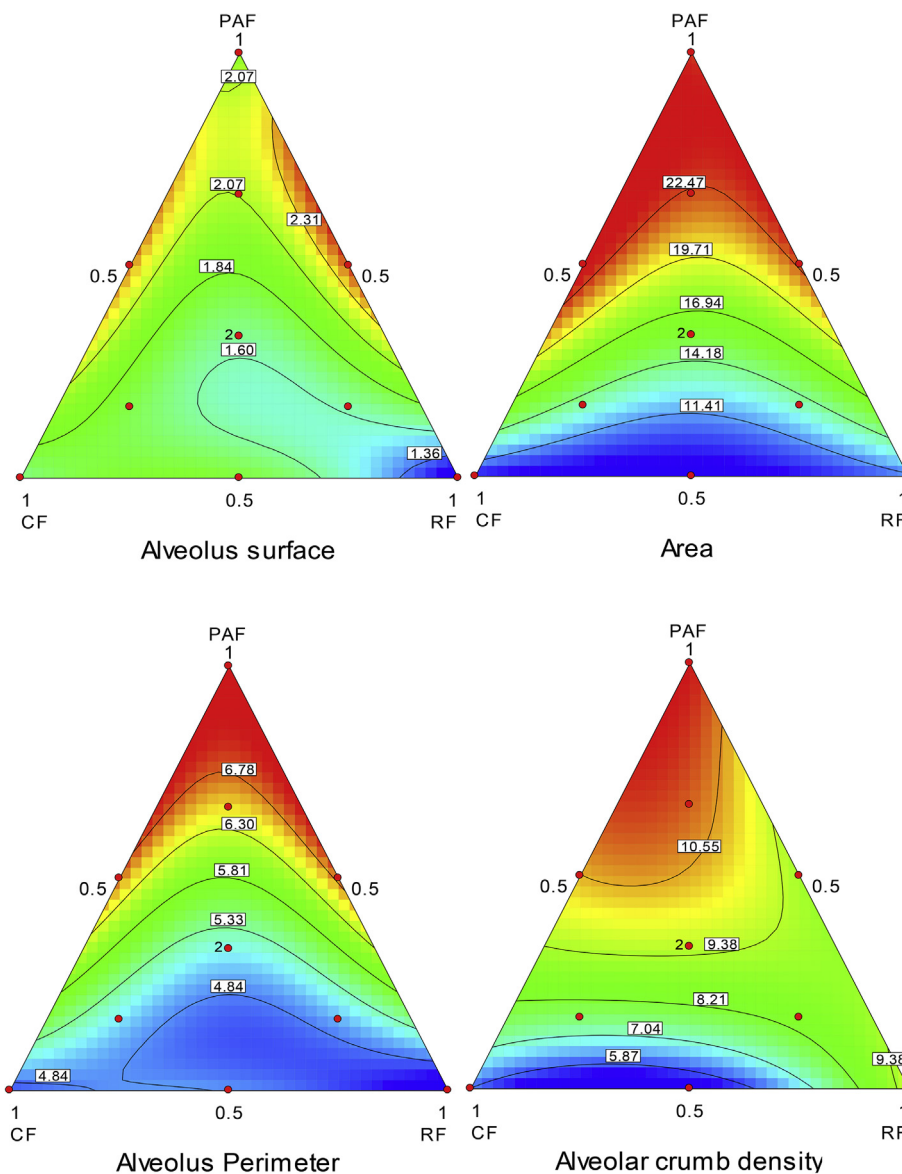


Fig. 4. Surface contour graph for alveoli parameters of muffin's crumb: (a) individual alveolus surface, (b) percentage of area occupied by alveoli, (c) alveolus perimeter, (d) alveolar crumb density.

density and total area occupied by alveoli previously described. Higher density and total area of alveoli lead to lower elastic crumbs with low chewiness. Gallagher, Gormley, and Arendt (2003) for gluten-free breads with lactic proteins of different sources did not find the same tendency. They found that area of alveoli and texture changed according to the type of protein incorporated to bread. Hager and Arendt (2013) for gluten-free breads prepared with rice flour, buckwheat and corn described a similar behavior found in this work. Hager et al. (2012) did not found any correlation between alveolar density and hardness for different breads with and without gluten. Nunes, Moore, Ryan, and Arendt (2009) proved different emulsions for a gluten-free mixture and did not found relationship between alveolar density and hardness of crumbs. Therefore, all these research suggest that not always a high alveolar density leads to low chewiness; this relationship depends on mixtures used in breadmaking.

Cohesiveness is a texture parameter related to the ability of the structural components of the crumb to remain assembled contributing to a lesser crumbling against the application of a force. Values of cohesiveness did not experienced great variations between different mixtures; they were in the range of 0.415 (M1)-0.478 (M2). The flour

with the highest capacity of obtaining a more cohesive crumb was RF, while that with the lowest cohesiveness properties was CF, presenting muffins with PAF intermediate values (Fig. 5).

Unlike wheat bread, these muffins prepared with PAF, CF and RF presented crumbs with certain adhesiveness. This behavior is related with the presence of milk and egg on muffin's mixture that are contributing with lipids that acts as lubricants. On the other hand, these flours do not form gluten but they contain hydrocolloids that retain high quantities of water, and even water is retained in different manners by each gluten-free flour. Muffins with PAF presented the more adhesive crumbs (M3 and M9), suggesting that less quantity of water was incorporated into the structure, probably due to the high content of insoluble fiber and sucrose. Muffins with RF presented very low values of adhesiveness, being M2 (only RF) the sample with the lowest value of this parameter (Fig. 5 blue zone).

All parameters described were adjusted at $p < 0.05$ level to quadratic models with r^2 higher than 0.94. Equations obtained with models were the following:

$$\text{Chewiness} = 7.73 \text{ CF} + 7.52 \text{ RF} + 2.39 \text{ PAF} - 0.75 \text{ CF.RF} - 8.35 \text{ CF.PAF} - 6.22 \text{ RF.PAF} \quad (p_{\text{lack fit}} = 0.877, p_{\text{model}} = 0.0013, r^2 = 0.96)$$

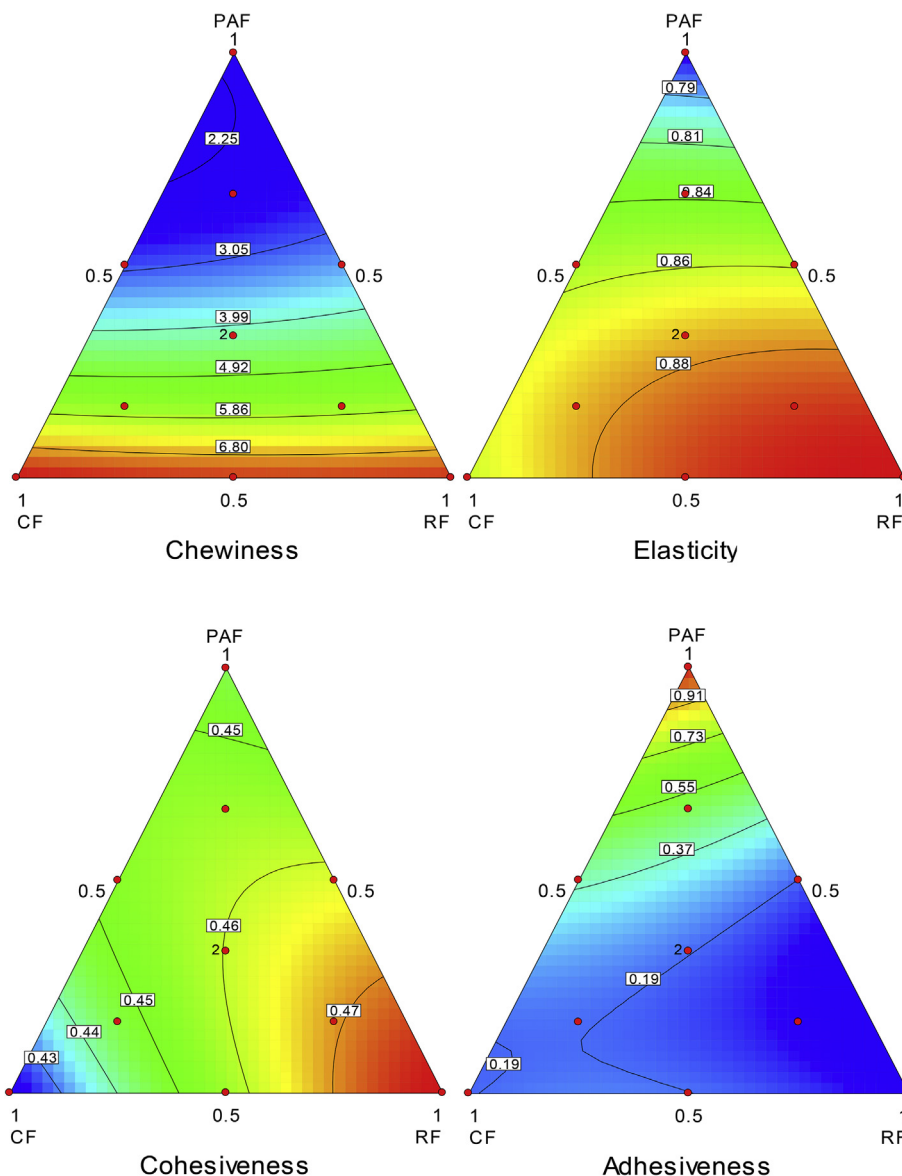


Fig. 5. Surface contour graph for texture parameters of muffin's crumb: (a) chewiness, (b) elasticity, (c) cohesiveness, (d) adhesiveness.

Table 2
Sensory attributes of quality of muffins.

Sample	Overall Acceptability	Color	Flavor	Taste	Chewiness
5	6.05 ± 1.76 ^B	6.66 ± 1.55 ^A	6.21 ± 1.83 ^A	5.51 ± 2.08 ^B	3.32 ± 1.54 ^A
6	6.30 ± 1.77 ^{AB}	6.70 ± 1.44 ^A	6.30 ± 1.70 ^A	5.84 ± 2.01 ^{AB}	3.18 ± 1.42 ^A
9	6.62 ± 1.74 ^A	6.78 ± 1.58 ^A	6.62 ± 1.68 ^A	6.38 ± 1.82 ^A	2.93 ± 1.55 ^A
10	6.05 ± 1.50 ^B	6.45 ± 1.46 ^A	6.32 ± 1.62 ^A	5.75 ± 1.71 ^B	3.39 ± 1.34 ^A

Different letters in the same column indicate significant differences between values (p < 0.05).

$$\text{Elasticity} = 0.86 \text{ CF} + 0.90 \text{ RF} + 0.76 \text{ PAF} + 0.03 \text{ CF.RF} + 0.16 \text{ CF.PAF} + 0.09 \text{ RF.PAF} \text{ (} p_{\text{lack fit}} = 0.782, p_{\text{model}} = 0.0004, r^2 = 0.97\text{)}$$

$$\text{Cohesiveness} = 0.41 \text{ CF} + 0.47 \text{ RF} + 0.43 \text{ PAF} + 0.029 \text{ CF.RF} + 0.091 \text{ CF.PAF} + 1.3\text{E-}003 \text{ RF.PAF} \text{ (} p_{\text{lack fit}} = 0.696, p_{\text{model}} = 0.0009, r^2 = 0.96\text{)}$$

$$\text{Adhesiveness} = 0.18 \text{ CF} + 0.04 \text{ RF} + 1.09 \text{ PAF} + 0.29 \text{ CF.RF} - 1.00 \text{ CF.PAF} - 1.53 \text{ RF.PAF} \text{ (} p_{\text{lack fit}} = 0.952, p_{\text{model}} = 0.0039, r^2 = 0.94\text{)}$$

3.6. Sensory properties of muffins

Average values of each sensory attribute of the muffins with the highest content of *Prosopis alba* flour (M3, M9, M5 and M6) are shown in Table 2. Only overall acceptability and taste presented significant differences between mixtures, presenting sample M9 the highest punctuation for these parameters with values significant different from those observed for M5 and M10, although similar to M9. Even though

Table 3
Nutritional composition of muffins.

g/100 g muffin (d.b.)	Muffins			
	5	6	9	10
Proteins	14.5 ± 0.40 ^B	12.8 ± 0.40 ^A	14.4 ± 0.20 ^B	14.5 ± 0.20 ^B
Lipids	15.6 ± 0.80 ^A	15.0 ± 2.0 ^A	13.0 ± 3.0 ^A	15.0 ± 2.0 ^A
Ash	3.5 ± 0.4 ^C	3.2 ± 0.4 ^B	3.4 ± 0.5 ^C	3.0 ± 0.3 ^A
Total Dietary Fiber	9.4 ± 0.9 ^B	7.0 ± 0.1 ^A	9.7 ± 0.7 ^B	5.7 ± 0.4 ^A
Soluble reducing sugars*	12.7 ± 0.5 ^A	15.4 ± 0.1 ^B	15.6 ± 0.1 ^B	15.0 ± 1.0 ^B
Sucrose	21 ± 3 ^B	17.2 ± 0.03 ^{AB}	22 ± 0.3 ^B	14 ± 0.1 ^A
Fructose	6 ± 1 ^A	5.3 ± 0.4 ^A	5.0 ± 0.1 ^A	5.1 ± 0.2 ^A
Glucose	6 ± 1 ^A	4.7 ± 0.4 ^A	4.1 ± 0.2 ^A	4.6 ± 0.2 ^A
Polyphenols	0.28 ± 0.03 ^C	0.23 ± 0.02 ^B	0.295 ± 0.006 ^C	0.195 ± 0.004 ^A
Antioxidant Activity**	1810 ± 80 ^C	1600 ± 100 ^B	2490 ± 90 ^D	1180 ± 20 ^A

*Somogyi-Nelson, **TEAC, mmol Trolox/100 g sample (d.b.).

Different letters in the same raw indicates significant differences between values ($p < 0.05$).

color, aroma and chewiness did not present significant differences, a clear tendency to higher values of M9 was observed (Table 2). In the case of chewiness, this attribute is satisfactory if low values are detected. All mixtures presented low values of chewiness, less than 3.4 that correlated with chewiness measured by texture profile analysis (Fig. 5). Zolfaghari, Harden, and Huffman (1986) for muffins with wheat flour replaced with different percentage of *Prosopis glandulosa* flour evaluated sensory attributes using a hedonic scale of 10 points, with value 0 as “unacceptable” and 10 as “excellent”. These authors found for color and taste values between 7.4 and 7.2. The fact that no significant differences were detected in color, aroma and chewiness parameters between samples, suggest that from the point of view of the consumer's acceptability it is possible to conclude that *Prosopis alba* flour can be included with corn and rice flours in muffin's mixtures without changing their sensory attributes.

3.7. Nutritional properties of muffins

Table 3 shows different nutritional attributes of the muffins containing the highest amount of *Prosopis alba* along with corn, rice or corn +rice flours (M5, M6, M9 and M10). The muffin with the highest content of PAF (M9) of the four formulations selected, presented high quantity of protein, ash and total dietary fiber (TDF) and low of lipids, with no significant difference with muffin M5. The central point (M10) presented the lowest content of total dietary fiber (TDF), behavior followed by M6 and M5-M9 in equal conditions. Results suggest that both PAF and CF contributed to dietary fiber. PAF and CF incorporated soluble reducing sugars to muffins that contribute to the Maillard reactions and brownish color development. The level of sucrose was significantly higher in M5, M6 and M9, indicating that PAF is the main flour that contribute with this non-refined sugar. No significant differences were observed for fructose and glucose proportions between the different muffins. Regarding to polyphenols, muffin M9 presented the highest amount, due to the high level of these bioactive substances in PAF. Muffin M9 also presented the highest antioxidant activity, significantly higher than that observed for sample M5, in spite of they present the same level of polyphenols. This behavior could be attributed to the fact that PAF present other components different from polyphenols with high antioxidant capacity.

4. Conclusions

Gluten-free muffins with American carob flour presented different physical (volume, color, texture) and sensory (taste, overall acceptability) characteristics, better than those found in products formulated with only corn or rice flour. The bread with the highest content of *Prosopis alba* flour developed the less chewiness and greatest adhesiveness that confers moisture sensation to the product. These attributes

are straightforward related to crumb structure: although more irregular alveoli occupied a greater area proportion, with a higher alveolar density in muffins with *Prosopis alba* flour, leading to softer crumbs. On the other hand, the greatest score for overall acceptability and the lower value of chewiness of the sample with American carob flour (M9), ratify the great potential of this flour as gluten-free ingredient for breadmaking, coming from an underutilized forestall species. From sensory point of view results evidence that attributes confer by *Prosopis alba* flour contributed in a positive form to muffin's quality; in addition, the more brownish color was well accepted. Besides, from the four muffins selected for sensory and nutritional analysis (M5, M6, M9 and M10), that with the highest level of *Prosopis alba* flour (M9) presented an adequate amount of protein (> 14%), the lowest amount of lipids and the highest percentage of dietary fiber, polyphenols and antioxidant activity. Therefore, due to the presence of significant amounts of natural sucrose and bioactive molecules with antioxidant activity, this flour would be a suitable ingredient as mimetic of chocolate-based functional breads.

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