

## Research Article

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# Increase in the antioxidant content in biscuits by infusions or *Prosopis chilensis* pod flour

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**Abstract:** Nowadays there is an increasing demand for healthy biscuits. The reduction in sugar and fat level, as well as the addition of bioactive compounds, is positively associated with a healthy diet. In the present work, low-fat and low-sugar biscuits were prepared with infusions (mate, coffee, and tea) or with *Prosopis chilensis* pod flour (PPF). Biscuits were made with maize starch and wheat flour (gluten formulations) or with gluten-free ingredients (gluten-free). The colour, texture, and the antioxidant capacity were evaluated in dough and biscuits. Among the formulations prepared with infusions, the mate dough showed the lowest firmness (1.1 N (gluten)-24.3 N (gluten-free)). However, no significant differences were found in the fracture stress of the final products ( $P > 0.05$ ). Mate gluten biscuits and PPF gluten-free biscuits showed the highest fracture strain (16.2 and 9.4%, respectively) and the lowest Young's modulus (7.3 and 13.3 MPa, respectively) in their groups. The highest antioxidant activity was found in biscuits with mate (8.7  $\mu\text{mol FeSO}_4/\text{g}$  (gluten)-4.3  $\mu\text{mol FeSO}_4/\text{g}$  (gluten-free)). These values were three times higher than the ones found in the control biscuits (2.9  $\mu\text{mol FeSO}_4/\text{g}$  (gluten)-3.9  $\mu\text{mol FeSO}_4/\text{g}$  (gluten-free)). The present results showed that the antioxidant content in biscuits could be successfully increased with infusion addition.

**Keywords:** low-fat biscuits, mate, tea, coffee, gluten-free

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## 1 Introduction

Biscuits are one of the best-selling food products in many countries. They have a long shelf life and, because a wide variety of ingredients can be incorporated into their formulation, they can be a useful tool to improve the nutritional quality in the consumer's diet (Guiné et al. 2020). Nonetheless, biscuits generally contain high fat levels often more than 30% and a high sugar amount rarely less than 40%; therefore, they are not considered as healthy food.

The development of healthy biscuits can include the reduction in their sugar or fat levels and the incorporation of different bioactive components. Some authors made healthy biscuits through the incorporation of composite flours (Saha et al. 2011; Chandra et al. 2015; Dhan-khar et al. 2019), fibre (Brennan and Samyue 2004; Aboshora et al. 2019; Diez-Sánchez et al. 2019), resistant starch (Aparicio-Saguilán et al. 2007; Laguna et al. 2011), natural and artificial sweeteners (Mosafa et al. 2017; Nakov et al. 2019), fruits (Pathak et al. 2018), spices (Klunklin and Savage 2018; Sandhya and Waghray 2018), or fat replacers (Colla et al. 2018).

On the contrary, the increase in the number of celiac patients increases the demand of gluten-free food. Although hydrocolloids or other additives are generally used to improve the physical characteristics of these food products, they may reduce the consumer's acceptance. Moreover, gluten-free products present a high proportion of carbohydrates but they are deficient in protein, fibre, minerals, and vitamins (Saturni et al. 2010; Koidis 2016). Thus, the addition of ingredients that provide all the nutrients necessary to maintain a balanced diet and, at the same time, reduce the fat and the sugar content of gluten-free products is a matter of striking significance.

Various flours and starches have been studied as ingredients to replace wheat flour in gluten-free foods. Algarrobo pod flour or Mesquite flour is a gluten-free flour obtained from the grinding of the whole mature fruit (pod) of algarrobo trees (*Prosopis* spp.). These species are widely distributed in a large part of the South American

territory, and it is a great food resource for humans and animals in arid and semi-arid regions of the world. Algarrobo pod flour is used for various purposes: food, wood, fodder, and also some ethnohistorical references have indicated their consumption by local indigenous people (Capparelli 2007; Capparelli and Prates 2015). Moreover, previous authors have indicated that algarrobo pod flour presents a high proportion of simple sugars and fibre, and also contains a significant amount of minerals, vitamins, and polyphenols with high antioxidant activity (Cardozo et al. 2010; Sciammaro et al. 2016; Gonzales-Barron et al. 2020).

It is well known that there is a positive correlation between food and health. Dietary guidelines worldwide recommend to increase the consumption of fruits and vegetables. These foodstuffs are rich sources of antioxidant and dietary fibre that could reduce the risk of human diseases such as cancer, atherosclerosis, heart diseases, osteoporosis, and obesity. Biscuits are often stored for extended periods; thus, antioxidant agents are one of the most used additives in the food industry. Natural antioxidants are generally preferred to potentially toxic, synthetic substances. Thus, several authors have studied the addition of natural antioxidant in biscuits with excellent results (Reddy et al. 2005; Mildner-Szkudlarz et al. 2009; Caleja et al. 2017). Furthermore, antioxidants can protect the body from oxygen radical-induced damage.

Infusions are natural aqueous extracts with high antioxidant content. Coffee (*Coffea arabica*) and tea (*Camellia sinensis*) are widely consumed beverages, and they are rich in bioactive phytochemicals such as chlorogenic acids, polyphenols, alkaloids, and melanoidins (Rodrigues and Bragagnolo 2013; Iriando-DeHond et al. 2019). Mate is an ancestral beverage consumed in several South American countries made from dried leaves of *Ilex paraguariensis*. Mate contains high levels of chlorogenic acids (Meinhart et al. 2018), saponins, purine alkaloids (Bracesco et al. 2011) vitamins, minerals, and several amino acids (Da Silva et al. 2008). Previous research has indicated the beneficial effects of mate consumption (Heck and Mejia 2007; Bracesco et al. 2011; Riachi and De Maria 2017; Gómez-Juaristi et al. 2018). Moreover, because of the long history of safe usage of these infusions and their high content of antioxidant substances, their addition into biscuit formulation is of particular interest to increase their nutritional value.

It is well known that the proportion of fat and sugar strongly influences the machinability of the dough as well as the quality of the finished product (Maache-Rezzoug et al. 1998; Baltsavias et al. 1999; Rodríguez-García et al. 2013). The fat acts as lubricant and contributes to dough plasticity, whereas the sugar contributes

to volume, colour, tenderness, sweetness, and also acts as preservative (Mamat et al. 2010). The reduction in sugar and fat content in biscuits results in structural, textural, sensory, and hedonic consequences (Pareyt et al. 2009). Accordingly, the production of healthy low-fat and low-sugar biscuits enriched with natural antioxidants could be a worthwhile alternative to increase the amount of antioxidant in the diet. Besides, this may also be an attractive option for consumers who are increasingly concerned about the choice of healthy foods. However, the addition of new ingredients into biscuit formulations may adversely affect the viscoelastic properties of dough and the quality of the final products. Therefore, any modification of the formulation must be thoroughly evaluated.

Thus, the aim of this article was to verify that (1) healthy additive-free low-fat and low-sugar biscuits with or without gluten can be made with a delicate texture by an appropriate selection of blends of different ingredients (flours and starches), and (2) the addition of infusions or algarrobo flour into biscuits could improve their antioxidant content without affecting their quality.

## 2 Materials and methods

*Prosopis chilensis* pod flour (PPF) (9.0% proteins, 4.6% lipids, 74% carbohydrates, 7.2% moisture, and 4.50% ash) was prepared by grinding whole pods of *Prosopis chilensis*. Other ingredients were purchased in a local market, such as wheat flour (ash content less than 0.65%, 10.1% protein, 14.7% moisture), rice flour (6% proteins, 1.2% lipids, 80% carbohydrates, and 2.4% fibre), chickpea flour (16% proteins, 9% lipids, 45% carbohydrates, and 15% fibre), cassava starch, maize starch, high oleic sunflower oil (Cañuelas, Argentina), sucrose, baking powder, vanilla, cocoa powder, black tea (La Virginia, Argentina), green tea (La Virginia, Argentina), yerba mate (Unión, Argentina), and coffee (La Morenita, Argentina). The chemical reagents were of analytical grade.

### 2.1 Selection of biscuit formulation by sensorial analysis

Dough was prepared with a kneading machine (Phillipp Cucina, Brazil) using a dough hook attachment at medium speed (837 rpm). First, the oil and the sugar were creamed during 30 s; then, the infusions or the water and vanilla

essence were added and mixed for 90 s. Finally, the mix of flour, starches, and baking powder was added and mixed for 120 s.

Dough was set into a polypropylene bag and held at 4°C for 30 min before it was extended with a rolling pin to give a thickness of 0.3 cm. Circular pieces of dough of 2.5 cm in diameter were cut and placed on a silicon sheet. The dough pieces were baked in an oven (Ariston type F9M, Italy) at 175°C with forced convection at different baking times.

To evaluate whether the addition of infusions (5%) could cause aromas, colours, or flavours perceived by consumers, two sensory tests were run. These assays were also used to optimize the proportion of ingredients that allowed the best texture in the low-fat and low-sugar biscuit. A total of 24 untrained panellists evaluated the colour, the texture, the aroma, the taste, and the global acceptance of four biscuits coded with random digits on a hedonic scale (1 = dislike and 10 = like very much).

The first sensory test was performed with gluten biscuit formulations made with four blends of wheat flour and maize starch (WF:MS) (100.0 g): 20.0 g of sugar, 8.0 g of high oleic sunflower oil, 1.0 g of vanilla essence, 1.0 g of baking powder, and 46.0 g of tap water. In three formulations, 30.0 g of water was replaced by 5% infusions (mate, coffee, and tea). Biscuits were baked for 22 min.

The second sensory test was made with four gluten-free blends with rice flour, cassava starch, maize starch, and chickpea flour (RF:CS:MS:CF) (100.0 g): 5.0 g sugar and 5.0 g PPF, 1.0 g of vanilla essence, 2.0 g of baking powder, and 35.0 g of tap water. In three of them, 30.0 g water was replaced by 5% infusions (mate, coffee, and tea). Biscuits were baked for 18 min.

Then, two new batches of biscuits were prepared using the formulations selected in the sensory tests: a control formulation (with water), formulations with 5% infusions (mate, coffee, black, or green tea), and one prepared with a mix of sugar and PPF (1:1). Gluten samples (formulated with a WF:MS mixture) were prepared with 46 g of tap water, while gluten-free samples (prepared with the RF:CS:MS:CF mixture) with 35 g. When PPF was added into formulations, a slightly higher amount of water was necessary to add (12 mL). Six rectangular pieces (5.0 × 2.5 × 0.3 cm) of gluten or gluten-free biscuit formulations were baked at 175°C during 32 min or 22 min, respectively.

## 2.2 Infusions' preparation

Coffee, black or green tea, and yerba mate infusions were prepared (5% w/v) with warm tap water (60°C) in a

thermostatic bath during 15 min. Then, the extracts were cooled at room temperature before their use. The pH of infusions was determined using a Mettler Toledo meter (SevenMulti, China) at 25°C.

## 2.3 Dough and biscuits characterization

Texture profile analysis (TPA) was performed with a texture analyser (TA-XT2i, Stable Micro Systems Ltd, England). The dough samples with a 2.0 cm diameter and 1 cm height were compressed and decompressed during two penetration cycles. Compression was exerted by a 7.5 cm diameter cylindrical probe with a test speed of 0.5 mm/s and with a 50 kg load cell. The strain was set at 50% and 30 s between cycles. Firmness (N), consistency (N s), adhesiveness (N s), springiness, and cohesiveness were calculated from the TPA plot (Gómez et al. 2007). The pH of dough was measured with an electrode for solid samples.

The fracture properties of the rectangular biscuits were studied by a three-point bending test performed with a TA.XT2 Texture Analyser (Stable Micro Systems Ltd, England), with trigger force of 25 g and load cell of 50 kg. Span length ( $L$ ) was 1.7 cm, and compression speed was set at 0.1 mm/s. Samples were placed on supports with their top surface down. The large, width ( $d$ ), and thickness ( $b$ ) of the baked products were measured using a Vernier calliper. The force ( $F$ ) needed to break the biscuit (N), the toughness or breaking work (N s), the deformation ( $y$ ) before rupture (mm), and the slope ( $s$ ) of force–distance curve (N mm) were determined. Texture of rectangular biscuits was expressed according to size-independent parameters (Baltsavias et al. 1999), and the fracture stress ( $\sigma$ , equation (1)), the fracture strain ( $\varepsilon$ , equation (2)), and the Young's modulus ( $E$ , equation (3)) were calculated as follows:

$$\sigma = \frac{3FL}{2db^2}, \quad (1)$$

$$\varepsilon = \frac{6by}{L^2}, \quad (2)$$

$$E = \frac{sL^3}{4db^3}. \quad (3)$$

The texture was measured 24 h after baking (to minimize the impact of moisture gradients in the baked product during cooling) with at least five different biscuits of each formulation. The water activity of biscuits was measured with AquaLab Serie3 (Decagon Devices, Inc., Pullman, WA; at 25°C) in duplicate. Besides, the moisture content was also determined in duplicate (AOAC 1984).

The surface colour of at least five samples (dough and biscuits) was measured using a Chroma meter CR-400 (Osaka, Japan) with D65 illuminant, 10° angle of vision. The colorimeter was calibrated using a standard white plate. The Hunter parameters  $L^*$  ( $L^* = 0$  [black],  $L^* = 100$  [white]),  $a^*$  ( $-a^* =$  green,  $+a^* =$  red), and  $b^*$  ( $-b^* =$  blue,  $+b^* =$  yellow) were determined. Moreover, the whiteness index (WI, equation (4)) (Zucco et al. 2011) and the colour difference ( $\Delta E$ , equation (5)) between samples and the control biscuits (note with  $\circ$ ) were calculated as follows:

$$WI = 100 - \sqrt{((100 - L^*)^2 + a^{*2} + b^{*2})}, \quad (4)$$

$$\Delta E = \sqrt{((L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2)}. \quad (5)$$

## 2.4 Extraction of antioxidant from dough and biscuits

The extraction of antioxidants was carried out in duplicate on ground and sieve samples (<500  $\mu\text{m}$ ) of biscuits, dough, and in some ingredients with a high proportion of bioactive compounds. The extraction was performed with warm water as described by Morales et al. (2009) with slight modifications. Briefly, 0.2 g of samples was weighed and extracted with 1.5 mL of distilled water (45°C) by stirring for 5 min (orbital shaker MS1, IKA, Brasil). Then, after 30 min of resting time at 4°C, samples were centrifuged for 10 min (Giumelli Z-127-D Centrifuge, Argentina). The residue was extracted one more time with 1 mL of warm water and centrifuged in the same conditions. Finally, the two aqueous extracts of each sample were combined and stored at  $-18^\circ\text{C}$  before use.

## 2.5 Ferric reducing antioxidant power

The ferric reducing antioxidant power (FRAP) of the samples was determined as described by Benzie and Strain (1996). Briefly, 0.2 mL of each extract was mixed with 1.8 mL of fresh prepared FRAP reagent and kept in the dark for 20 min. The absorbance was measured at 593 nm (UVmin-1240 spectrophotometer, Shimadzu, Jenck S.A., Kyoto, Japan) in clear samples. A calibration curve was made with ferrous sulphate ( $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ ) within the range of 0–1,000  $\mu\text{M}$ . The FRAP of the samples was expressed as  $\mu\text{mol FeSO}_4$  per g of dried sample (biscuits,

dough, or dried ingredients) or  $\mu\text{mol FeSO}_4$  per mL in infusion samples.

## 2.6 Data analysis

Data were statistically evaluated by analysis of variance (ANOVA) at a 0.05 significance level. The least significant differences (LSD) were calculated to compare the means at a level of 95% using the Fisher's test.

Non-parametric statistical tests were used when the variables did not comply with the assumption of homogeneity of variance. As recommended by García-Gómez et al. (2019), the differences in the sensory characteristics (colour, texture, taste, aroma, and global acceptance) were studied using Friedman's test at 95% level of confidence.

# 3 Results and discussion

## 3.1 Selected formulation from sensorial analysis

The low-fat and low-sugar biscuits could be classified as semisweet biscuits according to Manley's classification (Manley 1998). The colour of the biscuits, the first characteristic that the consumers perceive and together with texture and taste, strongly affects the acceptability of the product. Data of colour, texture, and the sensory test of biscuits made with different ingredients are presented in Tables 1 and 2.

As presented in Table 1, a wide range of toughness values (196.2–625.1 N s) could be obtained from different wheat flour and maize blend composition. Biscuits made with 55:45 (WF:MS) with mate were preferred by panellist in texture, taste, and global acceptance ( $P < 0.05$ ). These samples showed the highest whiteness index (69.8) and a tender texture (low toughness, 196.2 N s)

On the contrary, the colour, the texture, and the sensory characteristics of gluten-free samples were also studied. According to the results presented in Table 2, no significant differences were found between samples in the sensory test ( $P > 0.05$ ). The highest toughness value (96.1 N s) and the lowest whiteness index (48.1) were registered in 26:12:12:50 (RF:CS:MS:CF) blend biscuits with high content of chickpea flour ( $P < 0.05$ ). Thus, the formulation 25:25:25:25 (RF:MS:CS:CF) was selected because it presented the highest scores in texture and global acceptance, besides it had a low value of toughness according to the bending test.

**Table 1:** Colour (whiteness index), texture (toughness), and results of the sensory test of biscuits prepared with wheat flour (WF) and maize starch (MS) of blends and with infusion addition

WF:MS blend ratio	Infusion	Whiteness index	Toughness (N s)	Sensory test				
				Colour	Aroma	Texture	Taste	Global acceptance
55:45	Mate	69.8 <sup>c</sup>	196.2 <sup>a</sup>	59.0 <sup>ab</sup>	58.5 <sup>a</sup>	77.0 <sup>b</sup>	73.0 <sup>b</sup>	74.0 <sup>b</sup>
62:38	Coffee	64.3 <sup>a</sup>	263.4 <sup>a</sup>	72.0 <sup>b</sup>	65.5 <sup>a</sup>	57.0 <sup>a</sup>	64.5 <sup>ab</sup>	63.0 <sup>ab</sup>
69:31	Tea	65.4 <sup>ab</sup>	380.4 <sup>ab</sup>	54.0 <sup>a</sup>	60.5 <sup>a</sup>	50.5 <sup>a</sup>	50.0 <sup>a</sup>	51.0 <sup>a</sup>
77:23	—	66.1 <sup>b</sup>	625.1 <sup>b</sup>	55.0 <sup>a</sup>	55.5 <sup>a</sup>	55.5 <sup>a</sup>	52.5 <sup>a</sup>	52.0 <sup>a</sup>

Means with different superscripts within the same column are significantly different ( $P < 0.05$ ).

Results showed that all gluten-free biscuits showed lower WI values than gluten samples because of the colour of CF (88.60L\*, -0.48a\*, 22.86b\*) and PPF (74.96L\*, 3.87a\*, 28.65b\*) (Table 2).

It should be highlighted that in both sensory tests, biscuits made with a high starch proportion were preferred by the panellists. Besides, among the formulations with infusion additions, biscuits made with mate received the highest taste score.

### 3.2 Infusion preparation

Previous authors studied the effect of infusion preparation conditions and its antioxidant content (Richelle et al. 2001; Sánchez-González et al. 2005; da Silveira et al. 2014). Mate extracts contain purine alkaloids (methyl xanthines), flavonoids (rutin), vitamins (such as vitamin A, B complex, C, and E), tannins, chlorogenic acid and its derivatives, and numerous triterpenoid saponins derived from ursolic acid (Bracesco et al. 2011). Green tea is made by inactivating the enzymes in the fresh leaves, and it contains flavonoids derivatives of catechins (monomers), whereas black tea contains more complex polyphenols (dimers and polymers) (Wang and Ho 2009). Coffee contains phenolic compounds such as chlorogenic acids, caffeine, and diterpenic compounds (Yashin et al. 2017).

Generally, coffee and tea are prepared with very hot water (90–95°C), while mate is prepared at 60–85°C. In this study, all infusions were prepared with tap water at 60°C. Mate infusion had pH 6.30, coffee pH 5.62, green tea pH 5.71, and black tea pH 5.17. The pH value measured in the coffee infusion is within the range of 5–5.8 reported by Derossi et al. (2018).

### 3.3 Dough characterization

Results of colour and dough texture of gluten samples are presented in Tables 3 and 4. In both the tables, the highest whiteness index was found in the control samples ( $P < 0.05$ ), while the highest colour difference was observed in the coffee samples ( $P < 0.05$ ). The colour differences between samples and control dough were less in the gluten-free samples because of the colour of the chickpea flour. The addition of mate infusion increased the pH of the dough, whereas with other infusions or with PPF addition, the values were lower than the control.

No significant differences were found between green tea, coffee, and control sample (Table 3). Mate sample shows the highest cohesiveness but also the lowest firmness and consistency values ( $P < 0.05$ ). Except for the PPF dough, all samples were prepared using the same quantities of ingredients (water, sugar, oil, and proteins) but

**Table 2:** Colour (whiteness index), texture (toughness), and results of the sensory test of gluten-free biscuits prepared from four blends of rice flour (RF), cassava starch (CS), maize starch (MS), and chickpea flour (CF) with infusions

RF:CS:MS:CF blend ratio	Infusion	Whiteness index	Toughness (N s)	Sensory test				
				Colour	Aroma	Texture	Taste	Global acceptance
25:25:25:25	Mate	57.2 <sup>c</sup>	42.8 <sup>a</sup>	67.0 <sup>a</sup>	64.5 <sup>a</sup>	64.5 <sup>a</sup>	71.0 <sup>a</sup>	67.0 <sup>a</sup>
38:12:12:38	Tea	54.4 <sup>b</sup>	53.2 <sup>a</sup>	60.0 <sup>a</sup>	57.0 <sup>a</sup>	55.0 <sup>a</sup>	56.5 <sup>a</sup>	58.5 <sup>a</sup>
50:12:12:26	Coffee	56.0 <sup>bc</sup>	46.1 <sup>a</sup>	65.5 <sup>a</sup>	63.5 <sup>a</sup>	57.0 <sup>a</sup>	54.5 <sup>a</sup>	59.5 <sup>a</sup>
26:12:12:50	—	48.1 <sup>a</sup>	96.1 <sup>b</sup>	47.5 <sup>a</sup>	55.0 <sup>a</sup>	63.5 <sup>a</sup>	58.0 <sup>a</sup>	55.0 <sup>a</sup>

Means with different superscripts within the same column are significantly different ( $P < 0.05$ ).

**Table 3:** Physical characteristics of dough and biscuits prepared from 55:45 wheat flour:maize starch blend flour with infusions or with *Prosopis chilensis* pod flour (PPF)

	Control	Coffee	Mate	Green tea	PPF
Firmness (N)	1.5 <sup>b</sup>	1.3 <sup>ab</sup>	1.1 <sup>a</sup>	1.4 <sup>b</sup>	4.8 <sup>c</sup>
Consistency (N s)	8.4 <sup>b</sup>	8.4 <sup>b</sup>	7.0 <sup>a</sup>	8.5 <sup>b</sup>	32.1 <sup>c</sup>
Cohesiveness	1.1 <sup>b</sup>	1.2 <sup>b</sup>	1.4 <sup>c</sup>	1.1 <sup>b</sup>	0.3 <sup>a</sup>
Springiness	0.98 <sup>a</sup>	0.98 <sup>a</sup>	0.98 <sup>a</sup>	0.98 <sup>a</sup>	0.98 <sup>a</sup>
Whiteness index, WI	74.1 <sup>d</sup>	59.4 <sup>a</sup>	66.4 <sup>b</sup>	68.9 <sup>c</sup>	67.0 <sup>b</sup>
Colour differences, $\Delta E$	—	15.4 <sup>c</sup>	11.3 <sup>b</sup>	9.9 <sup>a</sup>	10.2 <sup>a</sup>
pH	6.56	6.41	6.73	6.62	6.40

Means with different superscripts within the same row are significantly different ( $P < 0.05$ ).

different infusions; therefore, the differences in dough texture and dough pH could be related to the composition of the aqueous extracts. The texture of the sample with PPF showed the lowest cohesiveness and the highest firmness and consistency values ( $P < 0.05$ ). The replacement of sugar by PPF increases the amount of proteins and fibre in the formulation, and therefore, more water was required to obtain a homogeneous dough. Other authors reported a similar trend when replacing wheat flour with algarrobo flour (Bigne et al. 2016).

In Table 4, the control sample showed highest firmness and consistency values ( $P < 0.05$ ), while the mate sample showed the lowest values. Adhesiveness of samples with infusion addition was higher than control formulations ( $P < 0.05$ ). According to the results, it could be concluded that the replacement of half of sugar by PPF produced dough with less firmness, consistency, and springiness than control sample but with similar cohesiveness and adhesiveness values.

### 3.4 Quality properties of biscuits

Baking is a complex process, which includes evaporation of water, denaturation of proteins, starch gelatinization, and also Maillard reactions. Results of colour, humidity, dimensions, and fracture texture of biscuits are presented in Table 5 (gluten samples) and Table 6 (gluten-free samples). Although all the samples were baked simultaneously and had the same amount of ingredients (except for PPF), some unexpected differences were found in the moisture and water activity values. PPF samples showed a higher humidity and higher  $a_w$  values than control samples, probably because they were prepared with a

**Table 4:** Physical characteristics of gluten-free dough prepared from 25:25:25:25 rice flour:cassava starch:maize starch:chickpea flour blend with infusions or *Prosopis chilensis* pod flour (PPF)

	Control	Coffee	Mate	Black tea	PPF
<b>Dough</b>					
Firmness (N)	42.3 <sup>d</sup>	30.3 <sup>b</sup>	24.3 <sup>a</sup>	33.8 <sup>c</sup>	29.5 <sup>b</sup>
Consistency (N s)	212.2 <sup>d</sup>	156.6 <sup>b</sup>	129.6 <sup>a</sup>	181.9 <sup>c</sup>	162.3 <sup>b</sup>
Cohesiveness	0.100 <sup>a</sup>	0.113 <sup>c</sup>	0.105 <sup>b</sup>	0.101 <sup>ab</sup>	0.101 <sup>ab</sup>
Adhesiveness	2.2 <sup>a</sup>	4.0 <sup>c</sup>	3.9 <sup>c</sup>	3.0 <sup>b</sup>	2.3 <sup>a</sup>
(N s)					
Springiness	0.23 <sup>b</sup>	0.27 <sup>b</sup>	0.27 <sup>b</sup>	0.21 <sup>ab</sup>	0.15 <sup>a</sup>
Whiteness index, WI	64.7 <sup>d</sup>	58.5 <sup>a</sup>	61.9 <sup>c</sup>	61.3 <sup>bc</sup>	60.7 <sup>b</sup>
Colour differences, $\Delta E$	—	6.9 <sup>c</sup>	3.0 <sup>a</sup>	3.6 <sup>a</sup>	4.8 <sup>b</sup>
pH	6.81	6.61	6.91	6.70	6.62

Means with different superscripts within the same row are significantly different ( $P < 0.05$ ).

higher amount of water than the other formulations. In both tables, all the  $a_w$  values were lower than 0.8; therefore, it could be considered that the pathogen growth would be inhibited (Mauer and Bradley 2017). Biscuits with lower thickness also showed lower values of  $a_w$  and humidity and thus a crispy texture (high Young modulus values) ( $P < 0.05$ ). However, no significant differences were found in the fracture stress ( $\sigma$ ) values ( $P > 0.05$ ), indicating that all biscuits had similar hardness. Finally, it was observed that the lowest colour difference was observed in the mate samples (Table 5).

The control biscuit (Table 5) showed the lowest values of thickness,  $a_w$  and humidity, and the highest Young's modulus values (which indicates a crispy texture) ( $P < 0.05$ ). On the contrary, biscuits prepared with mate showed the highest fracture strain values ( $\epsilon$ ) and the lowest Young's modulus ( $P < 0.05$ ), indicating a tender texture. Other authors also found a reduction in the firmness texture with mate addition (Faccin et al. 2015). Mate biscuits also showed a significantly higher thickness, humidity, and  $a_w$  values ( $P < 0.05$ ). During baking, dough components (proteins, sugars, dietary fibre, and other high water affinity ingredients) are responsible for trapping water until it is released as a consequence of heating. Thus, differences in dough composition (extracts or PPF) produce that all biscuits retain more moisture than the control formulation. Regarding colour, coffee and PPF biscuits presented lower WI values and higher  $\Delta E$  values than other biscuits ( $P < 0.05$ ).

Results in Table 6 show that no significant differences were found in the thickness of gluten-free biscuits

**Table 5:** Physical characteristics biscuits prepared from the 55:45 wheat flour:maize starch blend flour with infusions or with *Prosopis chilensis* pod flour (PPF)

	Control	Coffee	Mate	Green tea	PPF
Fracture stress, $\sigma$ (kPa)	1.7 <sup>a</sup>	1.3 <sup>a</sup>	0.9 <sup>a</sup>	1.3 <sup>a</sup>	1.5 <sup>a</sup>
Fracture strain, $\epsilon$ (%)	5.0 <sup>a</sup>	8.2 <sup>a</sup>	16.2 <sup>b</sup>	7.9 <sup>a</sup>	6.8 <sup>a</sup>
Young's modulus, $E$ (MPa)	33.9 <sup>c</sup>	22.6 <sup>bc</sup>	7.3 <sup>a</sup>	17.8 <sup>ab</sup>	18.2 <sup>ab</sup>
Thickness (mm)	4.5 <sup>a</sup>	5.6 <sup>ab</sup>	6.7 <sup>b</sup>	5.6 <sup>ab</sup>	5.3 <sup>b</sup>
Moisture content (%)	6.7 <sup>a</sup>	8.7 <sup>c</sup>	11.2 <sup>d</sup>	7.9 <sup>b</sup>	9.0 <sup>c</sup>
$a_w$	0.399 <sup>a</sup>	0.559 <sup>b</sup>	0.672 <sup>c</sup>	0.487 <sup>ab</sup>	0.529 <sup>b</sup>
Whiteness index, WI	68.5 <sup>c</sup>	57.0 <sup>a</sup>	68.2 <sup>c</sup>	61.8 <sup>b</sup>	56.7 <sup>a</sup>
Colour differences, $\Delta E$	—	13.0 <sup>b</sup>	8.3 <sup>a</sup>	8.3 <sup>a</sup>	14.3 <sup>b</sup>

Means with different superscripts within the same row are significantly different ( $P < 0.05$ ).

( $P > 0.05$ ). Coffee biscuits showed the highest Young's modulus values (crispier texture) while samples with PPF showed the highest fracture strain (tender texture) ( $P < 0.05$ ). The colour analysis indicated that mate and PPF biscuits presented higher WI values and lower  $\Delta E$  values than coffee and tea biscuits ( $P < 0.05$ ).

It was observed that despite the differences in the texture of the dough with infusions or with PPF (Tables 3 and 4), there were no important differences in the texture of biscuits (Tables 5 and 6).

### 3.5 Ferric reducing antioxidant power

There are many different antioxidants, and it is very difficult to measure each antioxidant component separately. In this study, the FRAP assay was selected because it is simple, and the reaction is reproducible and linearly related to the concentration of the antioxidant(s) present. In the present study, antioxidants were extracted under the same conditions (warm water) and analysed together

with the dough and the biscuits samples by FRAP assay so that the results could be compared. The values found for these ingredients were ( $\mu\text{mol FeSO}_4/\text{g}$ ): 80.4 *Moringa oleifera* leaf powder; 1.9 dried cranberries (*Vaccinium macrocarpon*); 9.8 raisins (*Vitis vinifera* L.); and 5.5 dried plums (*Prunus domestica*).

Results of FRAP assay are presented in Table 7. Antioxidant capacity of dough depends on the amount of antioxidants added with ingredients. During baking, the outer layers of the dough are heated to 170°C, but in the inner layers, the temperature remains lower than 100°C. Decomposition of antioxidant during baking is partially compensated by the formation of Maillard products (melanoidins) which also possess antioxidant activity (Patrignani et al. 2021). Only samples made with PPF and with black tea showed a higher antioxidant activity in biscuits than dough. PPF and all infusions are shown higher FRAP values than control samples. The antioxidant activity of samples made with PPF or mate and green tea infusions was higher than samples made with coffee and black tea infusions. The same trend was observed between the antioxidant content of the ingredients. All

**Table 6:** Physical characteristics of gluten-free biscuits prepared from 25:25:25:25 rice flour:cassava starch:maize starch:chickpea flour blend with infusions or *Prosopis chilensis* pod flour (PPF)

	Control	Coffee	Mate	Black tea	PPF
Fracture stress, $\sigma$ (kPa)	0.8 <sup>a</sup>	1.1 <sup>a</sup>	0.9 <sup>a</sup>	1.1 <sup>a</sup>	1.2 <sup>a</sup>
Fracture strain, $\epsilon$ (%)	3.5 <sup>a</sup>	4.6 <sup>ab</sup>	6.5 <sup>b</sup>	4.9 <sup>ab</sup>	9.4 <sup>c</sup>
Young's modulus, $E$ (MPa)	18.8 <sup>a</sup>	37.6 <sup>b</sup>	17.2 <sup>a</sup>	27.7 <sup>ab</sup>	13.3 <sup>a</sup>
Thickness (mm)	5.0 <sup>a</sup>	4.6 <sup>a</sup>	4.9 <sup>a</sup>	5.0 <sup>a</sup>	5.2 <sup>a</sup>
Moisture content (%)	6.9 <sup>ab</sup>	6.1 <sup>a</sup>	9.8 <sup>c</sup>	7.7 <sup>b</sup>	11.1 <sup>d</sup>
$a_w$	0.469 <sup>a</sup>	0.453 <sup>a</sup>	0.609 <sup>c</sup>	0.525 <sup>b</sup>	0.650 <sup>d</sup>
Whiteness index, WI	59.0 <sup>c</sup>	52.9 <sup>a</sup>	60.0 <sup>c</sup>	56.4 <sup>b</sup>	58.5 <sup>c</sup>
Colour differences, $\Delta E$	—	7.7 <sup>b</sup>	3.1 <sup>a</sup>	5.5 <sup>ab</sup>	4.8 <sup>ab</sup>

Means with different superscripts within the same row are significantly different ( $P < 0.05$ ).

**Table 7:** FRAP results in samples prepared with 55:45 wheat flour:maize starch (WF:MS) blend or with 25:25:25:25 rice flour:cassava starch:maize starch:chickpea flour (RF:CS:MS:CF) blend with infusions or *Prosopis chilensis* pod flour (PPF)\*

		Control	Coffee	Mate	Black tea	Green tea	PPF
Ingredients		—	8.8 <sup>*</sup>	12.1 <sup>*</sup>	7.8 <sup>*</sup>	12.8 <sup>*</sup>	18.6
55:45 (WF:MS)	Dough	2.0 <sup>a</sup>	4.4 <sup>b</sup>	8.1 <sup>d</sup>	—	5.7 <sup>c</sup>	4.4 <sup>b</sup>
	Biscuit	2.9 <sup>a</sup>	4.5 <sup>b</sup>	8.7 <sup>d</sup>	—	5.1 <sup>bc</sup>	5.6 <sup>c</sup>
25:25:25:25 (RF:CS:MS:CF)	Dough	1.3 <sup>a</sup>	3.3 <sup>c</sup>	3.9 <sup>d</sup>	2.0 <sup>b</sup>	—	3.0 <sup>c</sup>
	Biscuit	1.8 <sup>a</sup>	3.3 <sup>b</sup>	4.3 <sup>e</sup>	3.1 <sup>b</sup>	—	3.8 <sup>c</sup>

Means with different superscripts within the same row are significantly different ( $P < 0.05$ ).

\* Results are expressed as  $\mu\text{mol FeSO}_4/\text{g}$  solid samples or  $\mu\text{mol FeSO}_4/\text{mL}$  in liquid ingredients.

ingredients showed a higher antioxidant activity than dried cranberries and dried plumbs. The antioxidant capacity of mate samples (8.1 (WF:MS) – 3.9 (RF:CS:MS:CF)) was three times greater than the control dough samples (2.2 (WF:MS) – 1.3 (RF:CS:MS:CF)) and almost double in biscuits samples ( $P < 0.05$ ). This is in accordance with the higher antioxidant activity of mate compared to tea infusions reported by other authors (Bravo et al. 2007; Heck and Mejia 2007; da Silveira et al. 2014). No differences were found between the antioxidant activity of coffee and PPF dough samples, but in biscuits, PPF showed higher values than coffee biscuits.

The sensorial test showed that the incorporation of the infusions at 5% level did not cause significant changes in appearance (aroma or colour) perceived by consumers, and it could be considered a positive aspect to keep the traditional aspect of the biscuits.

## 4 Conclusions

Biscuits are products worldwide appreciated and consumed by different categories of consumers, child, young, and adults. Therefore, the reduced sugar and fat content could have a great impact on health. Low-fat and low-sugar biscuits enriched with natural sources of antioxidants may be attractive for consumers who are increasingly concerned about the choice of healthy foods. Algarrobo pod flour could be obtained at home, with a simple ground process, and it is a gluten-free ingredient that could be used as a partial sugar replacer.

The use of infusions is an environment-friendly process to obtain natural food additives. This study revealed that both the use of infusions (aqueous extracts of coffee, tea, and mate) and the use of PPF as biscuit ingredients allowed to obtain products with a high antioxidant activity. The infusion preparation conditions (concentrations, temperature, and time) and level of incorporation

on biscuit formulation should be further investigated. In addition, the results indicated that healthier low-fat and low-sugar biscuits without artificial additives and with a delicate texture could be obtained by selecting the right mix of ingredients.

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## Appendix

1      2      3      4      5



Photo of gluten-free dough prepared from 25:25:25:25 rice flour: cassava starch: maize starch: chickpea flour blend flour with infusions or *Prosopis chilensis* pod flour (PPF) (1: black tea; 2: coffee; 3: control (prepared with water); 4: PPF; 5: mate).