Research Article

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Quality evaluation of gluten-free biscuits prepared with algarrobo flour as a partial sugar replacer

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Abstract: Algarrobo flour is a gluten-free flour obtained by grinding the whole fruit (pods) of *Prosopis chilensis*. Because of its taste, this flour could be used as a sugar replacer. Besides, it can improve the protein profile, and the antioxidant and fibre content of foodstuffs. This is of special interest in gluten-free products that are generally deficient in these nutrients. In the present work a total of eleven different gluten-free biscuit formulations with Algarrobo flour and different proportions of rice flour, chickpea flour, cassava starch, and maize starch were evaluated. The effects of each ingredient were analysed and the texture, colour, total dietary fibre content, antioxidant capacity, and sensorial acceptability associated with the addition of algarrobo flour as a partial sugar replacer were studied in detail in three selected formulations. Results showed that the dietary fibre content and the antioxidant capacity increased with the addition of algarrobo flour (60 and 20%, respectively). In addition, no significant differences were found in the sensory scores of biscuits prepared with sugar or with algarrobo flour ($P \geq 0.05$). It could be concluded that algarrobo flour could partially replace sugar in gluten-free biscuit formulations. Besides, its addition increases the fibre and antioxidant capacity of the product without significantly changing its optimum texture. This is a major achievement that could be the starting point for future research aimed to develop new and healthy gluten-free products.

Keywords: algarrobo, gluten-free, biscuit, fibre

1 Introduction

Biscuits are a well-accepted food product. They are consumed at any time of the day and because of their low moisture content, they have a long shelf life. Besides, the current interest of consumers in healthy foods that contribute to prevent or reduce the risk of chronic diet-related diseases has increased the tendency to develop new and healthy biscuit formulations.

Moreover, in recent years, the demand for gluten-free products has also risen as a result of the increase in the number of diagnosed celiac patients. This disease develops in genetically predisposed individuals who are exposed to gluten [1,2]. Usually gluten-free foods tend to have a pale colour, lower volume, and present a more brittle texture than products with gluten. Therefore proteins, hydrocolloids, emulsifiers, or their combinations are usually added as structuring agents in order to simulate the viscoelastic properties of gluten [3]. In particular, the texture of biscuits is strongly associated with the gelatinization of starch, the crystallisation of sugar, and the association of these components with the lipids in the food matrix provided by the wheat [4,5].

Gluten-free products are generally rich in carbohydrates but deficient in protein, fibre, minerals, and vitamins [6], and often present a high glycaemic index [5]. Thus, the reduction in the carbohydrate content, together with the increase in protein content and the incorporation of raw ingredients that provide all the nutrients necessary to maintain a balanced diet, would be the starting point to obtain good healthy gluten-free biscuits.

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Several flours and starches have been studied as ingredients to replace wheat flour in gluten-free foods. Each of these components influences the characteristics of the final product in a different way. Rice flour (RF) and chickpea flour (CF), as well as cassava starch (CS) and maize starch (MS), are common and inexpensive ingredients that could be combined to achieve a good quality gluten-free product.

Algarrobo flour (AF) is a gluten-free flour obtained by grinding the whole fruit (pods) of *Prosopis* trees (*Prosopis* spp.). This flour contains a high amount of simple sugars (more than 50%) and fibre (20%), and important amounts of proteins, minerals, and antioxidant compounds. Hence, it has been indicated that algarrobo flour could be used as a sucrose substitute in bakery products. Besides, its addition could improve the content of protein, antioxidants, and minerals in foods. Previous authors have reported the use of this type of flour to obtain bread, muffins, and snacks [7–12] but there is only scarce information about its use in biscuits [13].

Considering this, the first objective of this work was to study the effect of different starches (CS and MS) and flours (RF and CF) to obtain a good quality gluten-free biscuit with no additive addition. Then, in order to obtain a healthier product, this formulation was improved by partially replacing the sucrose with the AF.

2 Materials and methods

All reagents were of analytical or HPLC grade, as required. The basic ingredients for the gluten-free biscuit formulations were purchased from a local grocery. The composition of the RF used was 6% proteins, 1.2% lipids, 80% carbohydrates, and 2.4% fibre, while the composition of CF was 16% proteins, 9% lipids, 45% carbohydrates, and 15% fibre (information provided by the producer). The AF was prepared and characterised according to the methods detailed below.

2.1 Preparation and characterisation of AF

Prosopis chilensis pods were harvested in La Rioja (Argentina) and their weight and size were recorded. Then, pods were washed by immersing in water and dried for 6 h at 80°C in an oven with forced air convection (Ariston type F9M, Italy). The dried pods were ground (Moulinex multiprocessor, Brazil) and sieved (500 µm) to obtain the AF that was stored in a hermetic container at room temperature.

The moisture (determined by drying at 105°C up to constant weight), ash (determined by calcination at 550°C for 3 h up to constant weight of white ash, AACC 08-01) [14], protein (by Kjeldahl method N × 6.25), and lipid content (by Soxhlet extraction using petroleum ether as solvent) were determined in the AF. The carbohydrate content was estimated by difference.

2.2 Amino acid profile of AF

Amino acids were determined after acid hydrolysis using RP-HPLC (Waters Corporation, Massachusetts, USA) with D,L-α-aminobutyric acid as internal standard according to ref. [15]. Briefly, the AF sample was digested in 6 M HCl, in anaerobic conditions. Then, it was incubated at 110°C for 24 h. Finally, the sample was derivatised with diethyl ethoxymethylenemalonate. For the determination, an HPLC Waters 2998 detector of variable wavelength (Massachusetts, USA) was utilised at 280 nm. A reverse phase column C18 (Waters Spherisorb, 5 µm ODS2 4.6 × 250 mm) thermostated at 25°C was used for separation. Samples were eluted with a binary gradient solution with solvent (A) sodium acetate (25 mM) containing sodium azide (0.02% w/v) of pH 6.0 and solvent (B) acetonitrile in the conditions described by Alaiz et al. [15]. The quantification of the different amino acids was performed in duplicate by using calibration curves of authentic standards, and results were expressed as grams of amino acid per 100 g of protein. This information was used to determine the chemical score of each amino acid in the AF [16].

2.3 Dough preparation and characterisation

Eleven flour-starch blends (100 g each) were prepared by mixing gluten-free flours (RF and CF) and starches (MS and CS) in different proportions (Table 1). All formulations were made with high oleic sunflower oil (8 g), baking powder (1.0 g), sugar (10 g), AF (10 g), vanilla essence (2.00 mL), and a proper amount of tap water. A kneading machine (Phillip Cucina, Brazil) was used for dough preparation at medium speed (speed 3 = 837 rpm). First, oil and sugar were creamed for 0.5 min, then water was added, and all the ingredients were mixed for 1.5 min. Finally, the flour blend and the baking powder were added and mixed for another 2 min. The obtained dough samples

Table 1: Composition of blends (B) expressed as g/100 g

Formulation	S:F ratio	CS	MS	RF	CF	w
I	2	33	33	33	0	47
II	2	33	33	0	33	47
III	1	25	25	25	25	40
IV	1	50	0	25	25	40
V	1	25	25	0	50	42
VI	0.5	0	33	33	33	42
VII	0.5	33	0	33	33	42
VIII	0.3	25	0	50	25	35
IX	0.3	0	25	50	25	35
X	0.3	25	0	25	50	35
XI	0.3	0	25	25	50	35

S:F – starch:flour ratio; CS – cassava starch; MS – maize starch; RF – rice flour; CF – chickpea flour; w – tap water (mL) added to 100 g of blend

were stored at 4°C in individual polypropylene bags before sheeting with a rolling pin to obtain a desirable thickness (1 cm for texture measurements and 0.3 cm for baking biscuits).

The texture profile analysis (TPA) of dough (discs of 3.0 cm diameter and 1.0 cm thickness) was performed with a texture analyser (TA-XT2i, Stable Micro Systems Ltd, England). Compression was exerted with a 7.5 cm diameter cylindrical probe with a 50 kg load cell, at a test speed of 0.5 mm/s. The strain was set at 50%, and the time between cycles was 30 s. The firmness (F), cohesiveness, and adhesiveness of the dough were determined from the force–time curves of the TPA according to Bourne [17]. At least five discs per formulation were analysed.

2.4 Biscuit preparation and characterisation

Rectangular pieces of dough (5 cm × 2.5 cm × 0.3 cm) were baked in an electric oven (Ariston type F9M, Italy) at 175°C with forced convection for 20 min and then allowed to cool at room temperature. The width (d), length (l), and thickness (t) of the baked products were measured using a Vernier caliper.

Fracture properties of the biscuits were studied by a three-point bending test at room temperature with a texture analyser (TA-XT2i, Stable Micro Systems Ltd, UK, 50 kg load cell) [18]. The span length (L, cm) was 1.8 cm, and the compression speed was set at 0.1 mm/s. The texture was analysed 24 h after baking to minimise the impact of moisture gradients on the baked product during cooling and storage at room temperature. Five samples of each formulation were placed on supports with their top surface down, and the force (F, N) needed to break the biscuit, the deformation (y, cm) before rupture, and the slope of the force–distance curve (s, N/cm) were measured. These parameters, together with the width (d, cm), length (l, cm), and thickness (t, cm) were used to determine the fracture stress (σ, equation (1)) (MPa), fracture strain (ε, equation (2)) (%), and Young´s modulus (E, equation 3; MPa) as follows:

$$\text{Fracture stress: } \sigma(\text{MPa}) = \frac{15 \times F \times L}{d \times t^2}, \quad (1)$$

$$\text{Fracture strain: } \epsilon(\%) = \frac{60 \times t \times y}{L^2}, \quad (2)$$

$$\text{Young's modulus: } E(\text{MPa}) = \frac{25 \times s \times L^3}{w \times t^3}. \quad (3)$$

The water activity (a_w) of the biscuits was also measured following the dew point sensor methodology in AquaLab Series 3 equipment (Decagon Devices, Inc., Pullman, WA, USA), at 25°C in duplicate. The moisture content of the biscuits was estimated by weight loss in an oven at 105°C [19].

2.5 Colour characteristics

The surface colour of the ingredients (flours and starches), dough, and biscuits was measured using a Chroma Meter CR-400 (Osaka, Japan) with the standard illuminant C that corresponds to average daylight (not including ultraviolet wavelength region), and a 2-degree standard observer angle. The colorimeter was calibrated using a standard white plate. The CIELAB parameters L^*(0 [black], 100 [white]); a^* ($-a^*$ [green], $+a^*$[red]), and b^*($-b^*$[blue], $+b^*$[yellow]) were determined. Six determinations were performed for each sample. The whiteness index (WI, equation 4) and chroma (C, equation 5) were calculated in the dough samples and the biscuits. Also, the colour difference (ΔE, equation 6) between the dough and the biscuit and the browning index (BI, equation 7) of the biscuits were determined as follows [20,21]:

$$\text{WI} = 100 - \sqrt{[(100 - L^*)^2 + a^{*2} + b^{*2}]}\,\frac{1}{2}, \quad (4)$$

$$C = \sqrt{(a^{*2} + b^{*2})}, \quad (5)$$

$$\Delta E = \sqrt{((L_0^* - L_1^*)^2 + (a_0^* - a_1^*)^2 + (b_0^* - b_1^*)^2)}, \quad (6)$$

$$\text{BI} = \frac{100}{0.17} \times \left(\frac{a_1^* + 1.75L_1^*}{5.645L_1^* + a_1^* - 3.01b_1^*} - 0.31\right), \quad (7)$$

where subscripts 0 and 1 indicate the dough and biscuits, respectively.

2.6 Physicochemical characterisation, antioxidant determination, and sensory evaluation of the selected biscuits

Three blends were selected according to their bread-making performance and physicochemical characteristics for further analysis. These three formulations were prepared with sugar (s) or by partially replacing the sugar with AF (s + AF). The biscuit preparation, texture determination, and colour characterisation were performed as described in the previous section.

The extraction of the antioxidant components was performed on milled and sieved biscuits or their dough (mixture of ingredients). Briefly, 0.20 g sample was extracted with 1.5, 1.0, and 0.5 mL of warm deionised water (45°C) in successive steps. The mixture was vortexed for 5 min at 37°C and left to rest for 30 min at 4°C, and then centrifuged at 10,000×*g* (10 min). The three successive supernatants were collected and kept at −20°C until use. The antioxidant capacity of the extracts was determined by the ferric reducing antioxidant power (FRAP) method according to the method described by Benzie and Strain [22]. Briefly, 200 µL of samples were mixed with 1.8 mL of FRAP reagent, and the sample absorbance was measured at 593 nm, after 25 min. The total dietary fibre (AACC method 32-05.01, measured with the enzymatic kit K-TDRF 05/12-Megazyme International, Ireland) [14] and the pH value (Mettler Toledo pH meter, SevenMulti, China) of the biscuits were also determined.

The sensory acceptability of the samples with sugar or with sugar + AF was evaluated by 36 untrained panellists (70% women; 30% men, non-smokers, between 20 and 50 years old). Panellists were recruited in a well-ventilated room at 4 pm, the location had a proper lighting, and it was free from distractions, noise, and odours. Round (3 cm × 0.3 cm) biscuits baked at 175°C for 17 min were presented with three-digit codes in randomised order. Panellists were explained about the terminology used in the assay and they were asked to evaluate the colour, texture, taste, and general acceptability on a hedonic scale ranging from 1 (extremely dislike) to 10 (like it very much). Water was supplied so that the panellists could rinse their mouths between each sample. All the participants were informed about the general aim of the study and gave their consent prior to participation.

2.7 Statistical analysis

An analysis of variance (ANOVA) of the data was performed by using a SYSTAT statistical computer program. A least significant difference (LSD) test with a confidence interval of 95% ($\alpha = 0.05$) was used to compare the mean values. Also, Friedman non-parametric test was used to compare sensory ranked results. In all the cases p-values lower than 0.05 were considered statistically significant.

In order to evaluate the strength of the relations between the physical variables, Pearson's correlation coefficients were calculated. Positive Pearson's regression coefficients indicate direct correlations, while large regression coefficients suggest strong associations. Besides, in an attempt to summarise the high dimensional space of the data set, while preserving the maximum allowable variance, data were analysed by a principal component analysis (PCA). This statistical tool identifies similarities between the formulations and the associations between the parameters studied. Prior to the analysis, data were standardised and centred. The selection of the number of components was based on the Catell's scree graph of their eigenvalues. Besides, the distance matrix considering the Euclidean distances of the samples was calculated [23].

3 Results and discussion

3.1 Pod and AF characterisation

The mean dimensions of *Prosopis chilensis* pods were: 5.62 ± 1.48 cm length, 0.65 ± 0.14 cm thickness, and 3.55 ± 1.24 g weight. These values were lower than the ones determined in *Prosopis alba* pods by Sciammaro et al. [24]. The production rate of AF was 54.5% (expressed as dry pod weight) and its composition (expressed in g/100 g flour) was: 9.81 ± 0.55 proteins; 4.58 ± 0.27 lipids; 7.18 ± 0.35 moisture; 4.50 ± 0.02 ash, and 73.93 carbohydrates. The protein content of AF in the present study was similar to the one reported by Díaz-Batalla et al. [25] in *P. laevigata* mesocarp flour (10.5 ± 0.5), but slightly higher than the values reported by Estévez et al. [26] in *P. chilensis* flour (7.8 g on dry weight basis).

The amino acid profile of AF can be seen in Table 2. Glutamic acid, aspartic acid, and isoleucine were the most important amino acids in the AF (they represent 44.4, 20.7, and 9.4%, respectively of the total amino acid content in the sample). Besides, the present results

Table 2: Amino acid composition (g/100 g protein) of AF (Algarrobo flour, *P. chilensis*) compared to *P. laevigata* mesocarp flour, *P. alba* germ flour, rice flour (RF), chickpea flour (CF), and FAO reference

Amino acid	AF[1]	*P. laevigata*[2]	*P. alba*[3]	RF[4]	CF[5]	FAO[6]
Arginine (Arg)	1.8 ± 0.5					
Aspartic acid (Asp)	56.9 ± 9.3					
Glutamic acid (Glu)	26.6 ± 5.3					
Glycine (Gly)	2.5 ± 0.1					
Serine (Ser)	1.2 ± 0.1					
Tyrosine (Tyr)	4.1 ± 1.5					
Histidine (His)	2.3 ± 1.5 (1.5)	2.6 (1.7)	5.1 (3.4)	2.0 (1.4)	3.2 (2.1)	1.5
Isoleucine (Ile)	12.0 ± 0.6 (4.0)	2.8 (**0.9**)	3.5 (1.2)	3.9 (1.3)	4.8 (1.6)	3.0
Leucine (Leu)	6.5 ± 0.8 (1.1)	7.5 (1.3)	9.7 (1.6)	7.7 (1.3)	8.5 (1.4)	5.9
Lysine (Lys)	4.9 ± 0.2 (1.1)	5.4 (1.2)	5.9 (1.3)	3.2 (**0.7**)	7.0 (1.5)	4.5
Threonine ± (Thr)	4.0 ± 0.1(1.7)	3.5 (1.5)	3.7 (1.6)	3.4 (1.5)	3.0 (1.3)	2.3
Valine (Val)	1.4 ± 0.2 (**0.3**)	4.0 (1.0)	4.5 (1.1)	5.3 (1.3)	4.4 (1.1)	3.9
AAA*	6.5 (1.7)	6.9 (1.8)	9.7 (2.5)	11.4 (3.0)	9.0 (2.4)	3.8
SAA**	1.6 (**0.7**)***	2.6 (1.2)	6.0 (2.7)	4.8 (2.2)	1.7 (**0.8**)	2.2
Total essential amino acids	39.2	35.3	48.1	41.7	41.6	27.1

*aromatic amino acids (Phenylalanine [Phe] + Tyrosine [Tyr] + Tryptophan [Trp]).
**sulphur amino acids (Methionine [Met] + Cysteine [Cys]).
***Cys was not determined.
[1]Values are expressed as mean values ± standard deviation (n = 2). Chemical scores are shown between parentheses.
[2]Díaz-Batalla et al. [25].
[3]Mamone et al. [27].
[4]Amagliani et al. [28].
[5]Zia-Ul-Haq et al. [29].
[6]Scoring pattern (g/100 g protein) for adult (>18 age) – World Health Organization, and United Nations University (2007).
The bold values indicate the lowest chemical scores.

indicate that the AF had a higher content of isoleucine and a lower content of valine and sulphur amino acids than other Prosopis flours [25,27].

The chemical scores for essential amino acids showed that the limiting amino acids in the AF were valine and the sulphur amino acids (methionine and cysteine). In contrast, isoleucine, lysine, and sulphur amino acids were the limiting amino acids in *P. laevigata* mesocarp flour, RF, and CF, respectively, according to the data reported by Amagliani et al. [28] and Zia-Ul-Haq et al. [29] (Table 2).

3.2 Effect of flour blend composition on dough and biscuit texture

The texture parameters of the dough and biscuits were significantly affected by the blend composition ($P < 0.05$). A wide range of textures was recorded in the dough (9–27 N firmness; 2.6–10.7 N s adhesiveness; 0.08–0.21 cohesiveness) and also in the biscuit (0.47–2.2 MPa fracture stress; 5.1–29.4% fracture strain; 2.1–43.9 MPa Young´s modulus) (Table 3).

Results of the dough characteristics showed that the lowest firmness values were recorded in the formulations VI and VII (9.0 and 9.7, respectively); both formulations presented a starch:flour ratio of 0.5. Results of 1.0 starch:flour ratio dough (formulations III and V) revealed that CF addition increased the adhesiveness and cohesiveness. Moreover, in blends with 0.3 starch:flour ratio (formulations VIII–XI), the MS produced a dough with higher firmness than CS, while the CF produced a dough with higher adhesiveness and lower firmness than the RF. The texture of formulation III (with equal proportion of all ingredients) presented high firmness (27.7 N) and low cohesiveness and adhesiveness (0.10 and 2.6 N s, respectively).

The texture of the biscuits was analysed by a three-point bend test. The fracture stress of the biscuits was related to the biscuit hardness; the Young´s modulus was associated with the stiffness, while the fracture strain represents the compressibility of the biscuit [18]. The highest values of fracture stress were found in formulation VI (2.20 MPa) and VII (2.17 MPa), both with a starch:flour ratio of 0.5. On the other hand, the highest fracture strain values were found in formulation IV (29.4%); X (28.4%), and IX (22.0%). These formulations also showed a high moisture content (≤13%) and high weight (>9 g). These effects could probably be associated with the high proportion of CS (formulation IV) and CF (formulations X and XI) that these blends had. Previous authors have

Table 3: Dough (TPA) and biscuit (Three-point bending test) texture of gluten-free formulations

Formulations[1]							Dough[2]			Biscuits[2]						
B	S:F	CS	MS	RF	CF	w	F (N)	Ad (N × s)	Coh (–)	*σ* (MPa)	*ε* (%)	*E* (MPa)	*l* (cm)	*t* (cm)	*W* (g)	*M* (%)
I	2	33	33	33	0	47	12.9^{ab}	5.2^{ab}	0.14^{bc}	1.71	5.1^{a}	43.9^{d}	5.4^{ab}	0.4^{a}	5.3^{a}	3.5^{a}
II	2	33	33	0	33	47	16.8^{b}	5.1^{a}	0.13^{bc}	0.86^{ab}	16.0^{bc}	5.8^{a}	5.4^{ab}	0.6^{bc}	7.3^{b}	12.9^{ef}
III	1	25	25	25	25	40	24.7^{cd}	2.6^{a}	0.08^{a}	0.69^{a}	7.6^{ab}	10.5^{ab}	5.5^{bc}	0.6^{bc}	8.6^{cd}	9.1^{c}
IV	1	50	0	25	25	40	21.6^{bc}	2.9^{a}	0.10^{a}	0.47^{a}	29.4^{d}	2.7^{a}	5.6^{c}	0.7^{cd}	9.5^{d}	13.6
V	1	25	25	0	50	42	20.4^{bc}	8.0^{cd}	0.15^{c}	0.94^{ab}	8.7^{ab}	10.5^{ab}	5.4^{ab}	0.6^{bc}	8.8^{cd}	10.5^{d}
VI	0.5	0	33	33	33	42	9.0^{a}	7.0^{bc}	0.20^{d}	2.20^{c}	6.2^{ab}	30.6^{cd}	5.3^{a}	0.5^{ab}	6.6^{b}	8.1^{b}
VII	0.5	33	0	33	33	42	9.7^{a}	6.7^{bc}	0.19^{d}	2.17^{c}	6.8^{ab}	33.4^{cd}	5.3^{a}	0.5^{ab}	7.1^{b}	7.7^{b}
VIII	0.3	25	0	50	25	35	22.3^{bc}	3.0^{a}	0.10^{a}	1.35^{b}	8.3^{ab}	20.4^{bc}	5.4^{ab}	0.6^{bc}	8.7^{cd}	12.2^{e}
IX	0.3	0	25	50	25	35	27.0^{d}	3.1^{a}	0.11^{ab}	1.43^{b}	5.6^{ab}	28.6^{c}	5.4^{ab}	0.5^{ab}	7.9^{bc}	7.7^{b}
X	0.3	25	0	25	50	35	16.8^{b}	10.8^{d}	0.21^{d}	0.52^{a}	28.4^{d}	2.1^{a}	5.5^{bc}	0.8^{d}	11.3^{e}	13.2^{f}
XI	0.3	0	25	25	50	35	21.1^{bc}	10.7^{d}	0.19^{d}	0.51^{a}	22.0^{cd}	2.8^{a}	5.5^{bc}	0.7^{cd}	11.4^{e}	13.7^{f}
$LSD_{0.05}$							*4.4*	*2.5*	*0.03*	*0.66*	*10.6*	*14.4*	*0.1*	*0.1*	*1.0*	*0.8*

[1]Composition of formulations (B) expressed as g/100 g starch:flour ratio (S:F); CS – cassava starch; MS – maize starch; RF – rice flour; CF – chickpea flour; w – tap water added (mL) to 100 g of blend.
[2]Mean values (n = 6). Values within a column with same superscript letters are not significantly different using the LSD test (P < 0.05).

indicated that both the ingredients presented a high water-holding capacity [30,31]; the increase in biscuit thickness by CF addition was also reported by Kohajdova et al. [32]. The firmness, consistency, and fracture stress of the formulations X and XI with a high content of CF were in agreement with Mancebo et al. [33], who found that protein incorporation increased the dough consistency, and reduced the hardness and spreading of biscuits.

3.3 Colour characteristics of ingredients, dough, and biscuits

The gluten-free flours and starches used for biscuit preparation showed different colour parameters (Table 4). The colour parameters of the obtained AF were slightly different from the ones reported by Estévez et al. [26] (67.5 L^*, 6.5 a^*, and 23.7 b^*). Besides, the AF and CF presented higher b^* values than RF and the starches (CS and MS).

The colour parameters of the dough and biscuits were significantly affected by the formulation (P < 0.05) and mainly due to their different CF contents (Table 4). The highest WI and lowest C values in the dough were recorded in formulation I (2 starch:flour ratio without CF), while the lowest WI and highest C values were recorded in formulations X and XI (0.3 starch:flour ratio with high CF content). A similar tendency was observed in biscuit colour: the highest WI and lowest C values were recorded in biscuits with formulation I, while the lowest WI values were found in biscuits X and XI. The darker colour of the samples with CF addition was also reported by Mohammed et al. [31], who analysed the effect of the partial substitution of wheat flour with chickpea flour on breadmaking quality. These authors indicated that the colour of the crust and crumb got darker as the level of chickpea flour increased.

Nonetheless, it is important to underline that the highest ΔE value was obtained in formulation I, with no CF addition, while low values of this parameter were obtained when a high proportion of CF was added (such as formulations X and XI). The parameter ΔE is associated with the effect of the thermal treatment on the colour of the food samples. Therefore, this result seems to indicate that although the dough became darker with the presence of CF, the thermal treatment had a limited effect on the colour development of these biscuits, and cooked samples with a high proportion of CF were more similar to the uncooked dough than samples with a low proportion of this flour.

3.4 Physicochemical properties analysed by multivariate statistical analysis

For a proper interpretation of the results for the dough and biscuits, two different PCAs were performed (Figure 1). The results of moisture content, dimensions, and textural parameters of the dough and biscuits can be seen in Figure 1a. The first and second principal components described 61.4

Table 4: Colour properties of samples*

B	S/F	$^1L_0^*$	$^1a_0^*$	$^1b_0^*$	$^1WI_0$	$^1C_0$	ΔE	$^2L_1^*$	$^2a_1^*$	$^2b_1^*$	$^2WI_1$	$^2C_1$
I	2	65.5^{d}	1.5^{a}	14.2^{a}	72.7^{g}	14.3^{a}	39.9^{e}	37.1^{d}	4.0 a	16.2^{a}	34.9^{de}	16.7^{a}
II	2	76.7^{g}	1.8^{b}	17.5^{b}	64.7^{e}	17.7^{b}	36.2^{d}	33.7^{c}	4.1 a	18.2^{b}	31.1^{c}	18.7^{b}
III	1	70.6^{f}	1.7^{ab}	15.1^{a}	66.9^{f}	15.4^{a}	34.8^{d}	36.4^{d}	5.9 b	20.4^{c}	33.0^{cd}	21.3^{c}
IV	1	65.6^{d}	2.5^{d}	22.2^{d}	59.1^{c}	22.0^{d}	32.1^{c}	33.9^{c}	3.7 a	17.2^{ab}	31.6^{c}	17.6^{a}
V	1	65.5^{d}	2.2^{c}	17.9^{b}	61.1^{d}	17.8^{b}	25.9^{a}	40.6^{e}	4.4 a	23.6^{d}	36.0^{e}	24.0^{d}
VI	0.5	68.6^{e}	1.5^{a}	17.1^{b}	64.2^{e}	17.1^{b}	37.2^{d}	31.9^{bc}	5.6 b	20.9^{c}	28.5^{b}	21.6^{c}
VII	0.5	67.5^{e}	1.7^{ab}	16.3^{b}	63.5^{e}	16.4^{ab}	36.7^{d}	31.2^{b}	5.7 b	20.4^{c}	28.0^{b}	21.2^{c}
VIII	0.3	63.1^{c}	2.4^{cd}	19.8^{c}	58.0^{c}	19.7^{c}	31.3^{b}	32.3^{b}	6.3 b	20.4^{c}	29.0^{b}	21.4^{c}
IX	0.3	61.6^{b}	2.3^{cd}	22.2^{d}	55.6^{b}	22.1^{d}	26.8^{a}	35.2^{d}	5.7 b	21.0^{c}	31.6^{c}	21.8^{c}
X	0.3	54.0^{a}	4.3^{e}	26.9^{e}	46.6^{a}	27.3^{e}	25.9^{a}	29.3^{ab}	7.6 c	20.5^{c}	26.0^{a}	21.9^{c}
XI	0.3	53.9^{a}	4.3^{e}	27.9^{e}	46.0^{a}	28.0^{e}	26.7^{a}	28.5^{a}	7.6 c	20.0^{c}	25.3^{a}	21.5^{c}
LSD $_{0.05}$		*1.3*	*0.2*	*1.6*	*1.6*	*1.8*	*2.6*	*2.2*	*0.8*	*1.0*	*2.2*	*1.0*

$^1X_0$ Dough values (n = 6).
$^2X_1$ Biscuits values (n = 6).
Values within a column with same superscript letters are not significantly different using the LSD test (P < 0.05).
*Colour parameters of gluten-free flours and starches used in the present work AF: 74.96 L^*, 3.87 a^*, 28.65 b^*; CF: 88.60 L^*, -0.48 a^*, 22.86 b^*; RF: 94.54 L^*, −0.39 a^*, 6.71 b^*; MS: 99.58 L^*, −0.84 a^*, 4.68 b^*; CS: 98.31 L^*, 0.36 a^*, 2.20 b^*.

and 25.9% of the variance, respectively. Principal component 1 (PC1) was better associated with the parameters that described the baked product (biscuits) such as fracture stress, fracture strain, and Young's modulus, while PC2 was mostly related to the dough parameters (cohesiveness, adhesiveness, and firmness). Results showed that formulations VI and VII (S:F ratio = 0.5) were very similar (Euclidean distance = 0.45), both were associated with high values of fracture stress and Young´s modulus, and short length. Besides, as depicted in Figure 1a, the adhesiveness and cohesiveness observed in the dough of formulations VI and VII were similar to the ones in formulations XI and X (S:F ratio = 0.3) (both presented positive values of PC2). However, in contrast to blends VI and VII, formulations XI and X presented high values of fracture strain, moisture content, and high weight (associated with positive values of PC1). Formulations IX and VII were also very similar (Euclidean distance = 1.98); according to Figure 1a, these formulations could be characterised by intermediate values of fracture stress and Young's modulus. It is important to outline that the only difference in the formulations VI and VII, XI and X, IX and VII was their CS or MS addition. Therefore, it could be concluded that these ingredients do not significantly modify the texture, moisture, or dimensions of the biscuits.

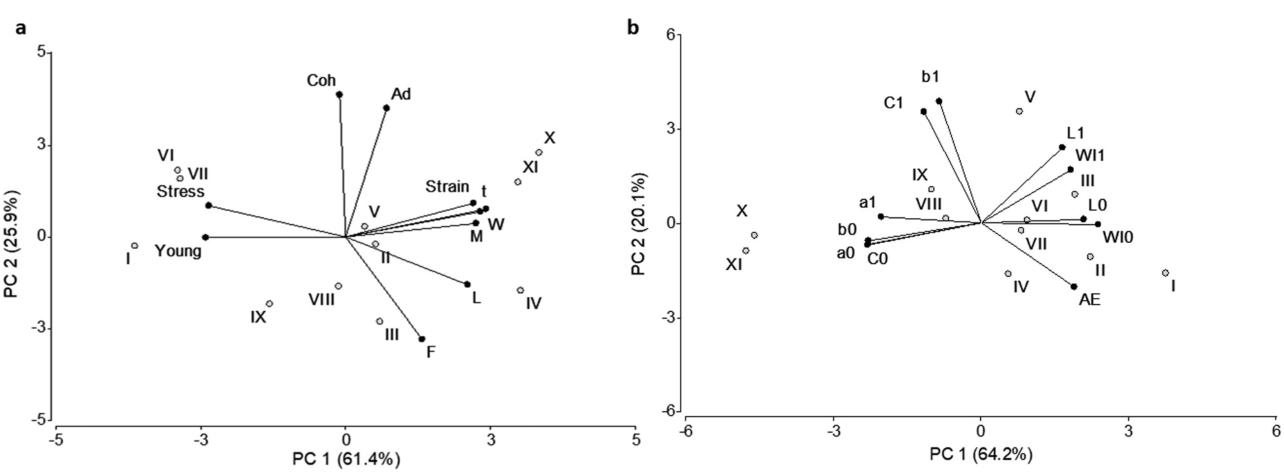

Figure 1: PCA of (a) the texture parameters (adhesiveness [ad]; firmness [F]; cohesiveness [Coh]; fracture stress [stress]; facture strain [strain]; and Young's modulus [Young]); dimensions (length [L]; thickness [t]; weigh [W]), and moisture content [M] and (b) colour parameters (L^*; a^*; b^*; chroma C, colour difference AE; and whiteness index WI) of dough and biscuit samples (subscript 0 and 1 indicated dough and biscuits, respectively).

Results also showed significant linear correlations ($P < 0.05$; Pearson's correlation coefficients >0.85) between the length of the biscuits and their fracture strain (correlation coefficient = −0.89), the thickness and the fracture strain (correlation coefficient = −0.87), the thickness and Young's modulus (correlation coefficient = −0.92), and between the moisture content and the Young's modulus (correlation coefficient = −0.91). This indicates that high Young's modulus values were recorded in low moisture and thin biscuits.

Another PCA was performed with the colour parameters of the dough and biscuits (Figure 1b). In this case, the first and second principal components described 64.2 and 20.1% of the variance, respectively. Opposite to the results previously described in Figure 1a, Figure 1b shows that PC1 was better associated with the parameters that described dough colour, while PC2 was related to the colour characteristics of the biscuits. Besides, as displayed in Figure 1b, formulations X and XI presented high values of b0 and a0, and low values of L0 and WI0. These formulations contained high proportions of CF, which reduces the luminosity of the samples. Moreover, results showed good correlations in the a^* values before and after the baking process ($P < 0.05$; Pearson's correlation coefficients = 0.71). This indicates that differences in the green/red colour of the samples are preserved in dough and biscuits.

3.5 Quality evaluation of selected biscuits

In this section, different formulations were selected to partially replace the AF with sugar. According to previous results, formulation IX (25MS:50RF:25CF) and XI (25MS:25RF:50CF) were selected because both had a high protein content but different texture characteristics. According to Figure 1a, formulation XI presents positive values of PC1 and PC2, while formulation IX has negative values in both the PCs. Besides, mixture III (25CS:25MS:25RF:25CF) was selected as it presents equal proportions of each ingredient.

The three selected formulations were prepared with sugar or with a partial replacement of sugar by AF (s + AF). The s + AF formulations presented a higher content of proteins and fibre; therefore, more water was required to obtain a homogeneous dough (in formulations prepared only with s, 35 g of water was added instead of 40 g for blend III and 27 g instead of 35 g for blends IX and XI). Results of the texture profile analysis of formulations III and XI showed that the firmness of dough pieces increased, whereas the adhesiveness and cohesiveness decreased because of the addition of AF to the formulations (Figure 2). On the other hand, the texture analysis of formulation IX showed that in this blend, only the firmness increased significantly with the AF addition ($P < 0.05$).

The highest values of firmness were recorded in blend XI made with s + AF (high CF content), in agreement with data reported by Aly and Seleem [34]. These authors also found that the hardness increased with the level of legume flour in bakery products.

The quality parameters of the biscuits were evaluated in terms of size (length [l], width [d], thickness [t], colour, and texture; Table 5 and Figure 3). No significant differences were found between the weight of the biscuits made with different blends and biscuits made only with sugar or with s + AF ($P \geq 0.05$). The results of the present work showed that the moisture of the biscuits was affected only by the blend composition (not by sugar replacement). The lowest values of moisture content were recorded in the biscuits from blend IX, due to the its low content of ingredients with high water binding capacity such as CF and CS. However, the final moisture content of the biscuits is not only associated with their composition, but also with the thermal treatment applied (time, temperature, and oven

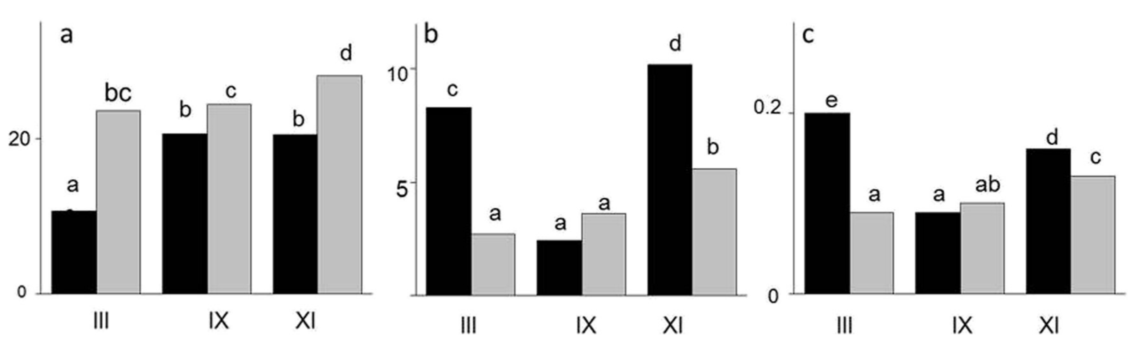

Figure 2: (a) Firmness (N); (b) adhesiveness (N × s); (c) cohesiveness (dimensionless) of dough prepared with formulations III, IX, or XI with sugar (black column) or with sugar and algarrobo flour (grey column).

Table 5: Colour and physical properties of selected samples made with s (sugar) or with s + AF (sugar and algarrobo flour)

Formulations	[1]III		[1]IX		[1]XI		
	s	s + AF	s	s + AF	S	s + AF	$LSD_{0.05}$
w (mL)	35	40	27	35	27	35	
[2]L_0*	78.7^e	73.6^d	73.8^d	70.4^c	68.4^b	64.8^a	0.5
[2]a_0*	−0.3^b	1.8^d	-0.5^a	1.7^d	1.4^c	3.0^e	0.1
[2]b_0*	19.7^c	18.4^b	19.8^c	16.3^a	22.1^d	18.3^b	0.8
[2]WI	70.9^e	67.7^d	67.2^d	66.1^c	61.4^b	60.2^a	0.7
[2]C	19.8^c	18.5^b	19.8^c	16.4^a	22.2^d	18.2^b	0.8
[2]L_1*	73.7^d	71.5^c	71.0^c	66.5^b	66.3^b	63.1^a	2.0
[2]a_1*	4.7^a	4.3^a	7.0^b	5.3^a	9.7^c	7.1^b	1.3
[2]b_1*	34.1^c	31.1^b	36.0^d	29.6^a	37.9^e	33.5^c	0.9
[2]C	34.4^c	31.4^b	36.7^d	30.1^a	39.2^e	34.3^c	1.0
Δ*E*	16.2^b	13.2^a	18.3^c	14.4^a	18.2^c	15.9^b	1.7
[2]WI	56.6^c	57.4^c	53.2^b	55.0^b	48.3^a	49.6^a	1.9
[2]BI	70.8^a	65.7^a	81.4^b	68.6^a	97.9^c	86.9^b	5.8
[2]M (%)	8.7^c	7.1^b	6.8^a	5.7^a	8.5^c	8.4^c	1.2
[2]l (cm)	5.50	5.59	5.46	5.52	5.49	5.50	>0.05
[2]d (cm)	3.00^a	3.1 ^{b}	3.13^b	3.11^b	3.17^b	3.17^b	0.07
[2]t (cm)	0.43	0.46	0.43	0.42	0.52	0.49	>0.05
[2]W (g)	5.70	6.31	6.03	6.55	7.01	6.60	>0.05
[2]pH	6.98^e	6.94^d	6.88^c	6.85^b	6.84^b	6.77^a	0.03
[2]a_w	0.39^a	0.59^b	0.41^a	0.59^b	0.44^a	0.62^b	0.13
[3]AA (μmol Fe^{2+}/g db)	35.0^a	57.4^b	36.2^a	68.3^d	32.5^a	63.1^c	5.1
[4]AA (μmol Fe^{2+}/g db)	55.8^a	75.2^c	65.0^b	84.7^d	59.3^a	80.7^{cd}	7.3
[4]Total dietary fibre (%)	4.0^a	7.7^b	4.5^a	8.2^b	6.5^a	10.6^b	3.1

[1]Selected biscuit formulations (g/100 g) III: 25CS:25MS:25RF:25CF; IX: 25MS:50RF:25CF, and XI: 25MS:25RF:50CF; CS – cassava starch; MS – maize starch; RF – rice flour; CF – chickpea flour.
[2]Dough or biscuits (n = 10).
[3]Mix of ingredients (n = 2) (dough).
[4]Ground biscuits (n = 2).
Values within a row with same superscript letters are not significantly different using the LSD test (P < 0.05).

characteristics) [4]. In the present work, only one cooking condition was analysed, thus only the effect of the composition could be properly analysed.

Besides, data showed that the AF addition significantly affected the pH and increased the a_w of biscuits (P < 0.05). Nonetheless, all a_w values were lower than 0.7, a cut-off value for pathogen growth [35]. Generally speaking, biscuits made with s + AF presented lower values of fracture stress (σ) and Young's modulus (E), and higher values of fracture strain (ε) than the samples prepared only with s; but only the increase in ε of biscuit III was significant (P < 0.05) (Figure 3).

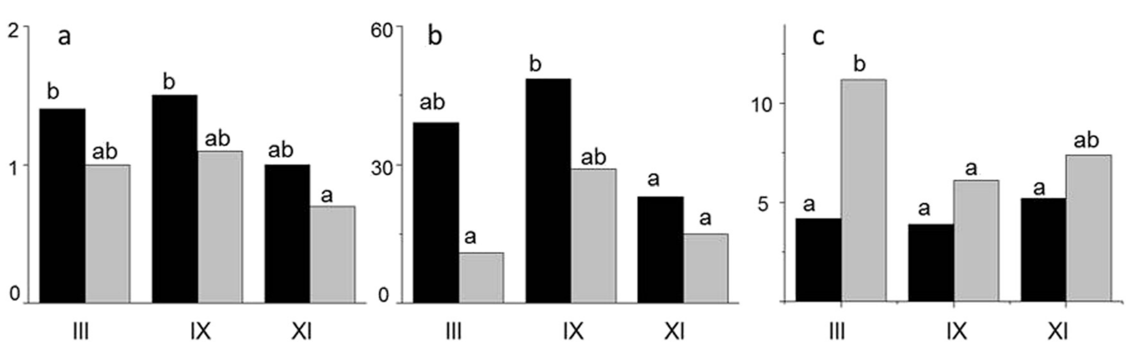

Figure 3: (a) Fracture stress (MPa); (b) fracture strain (%); (c) Young´s modulus (MPa) of biscuits prepared with formulations III, IX, or XI with sugar (black column) or with sugar and algarrobo flour (grey column).

Photographs of the selected dough and biscuits are depicted in Figure 4, and the colour parameters are listed in Table 5. In the case of dough, the addition of AF increased the a^* parameter (redder) and decreased L^*, b^*, C, and WI values compared to formulations prepared with sugar only. In line with this result, a decrease in L^* and b^* values was also recorded in the biscuits. The colour of the biscuits is related to the colour of the ingredients used in the formulations and also to the extent of Maillard reactions that take place during the baking process [36].

Results showed that the addition of AF decreased the ΔE (which indicates less colour changes during baking) and the browning index (BI) (less brown colour development) in formulations IX and XI ($P < 0.05$), but this effect was not significant in formulation III ($P \geq 0.05$). Other authors have reported similar colour modifications of products with AF such as bread and sweet snacks [8,12]. Furthermore, the antioxidant activity (AA) increased with the addition of AF to the formulations ($P < 0.05$). Results showed an increase of at least 30% in the samples with AF addition (dough and biscuits). Moreover, all the AA values were higher in the biscuits than in their respective dough. The AA increase after baking could be related to different factors: on the one hand, the heating procedure could have increased the accessibility of some compounds that could not be released before in the uncooked product because they were binding other compounds [37], on the other hand, the development of Maillard reactions could lead to the synthesis of compounds with anti-radical properties [38]. The high AA values observed in biscuits with AF could be associated with the presence of antioxidant components such as phenolic compounds, tannins, phenols, and flavonoids [37]. The highest AA value (84.7 µmol Fe^{2+}/g) was recorded in biscuits from blend IX s + AF (with high RF content).

The total dietary fibre content increased up to 50% with the addition of AF to the formulations. Results in Table 5 show that the fibre content in formulations III, IX, and XI increased from 4.0, 4.5, and 6.5% (g/100 g) to 7.7, 8.2, and 10.6%, respectively. Other authors have previously reported this increase in the fibre content due to AF incorporation in breads [8,9]. Therefore, the addition of AF to the formulation of gluten-free biscuits could be a healthy alternative to reduce its sugar content and, at the same time, increase its fibre content and antioxidant activity.

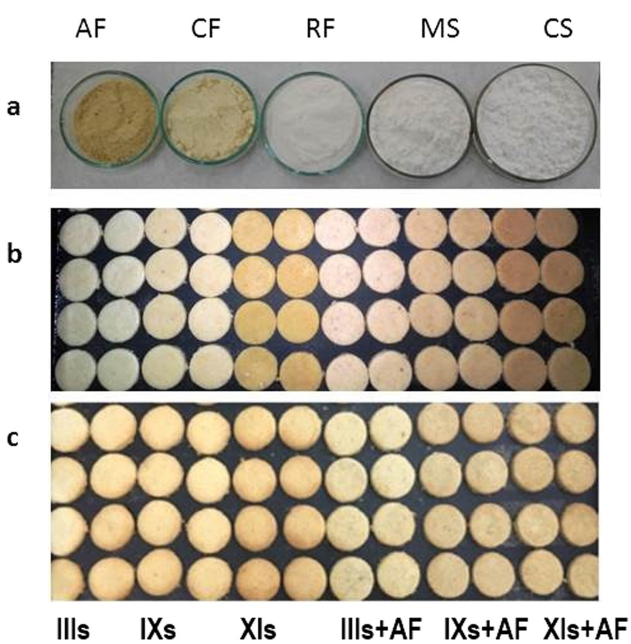

Figure 4: Photographs of (a) ingredients used in the preparations (AF – algarrobo flour; CF – chickpea flour; RF – rice flour; MS – maize starch; CS – cassava starch), (b) dough prepared, and (c) biscuits prepared with formulations III,IX, or XI with sugar (s) or with sugar and algarrobo flour [s + AF].

3.6 Sensory evaluation of selected biscuits

Results of sensory panels are presented in Figure 5. Significant differences were only found in the texture of the biscuits without AF, and the best score was recorded in biscuits XI ($P < 0.05$). Considering that the assayed biscuits were low in sugar and lipid content, and that the

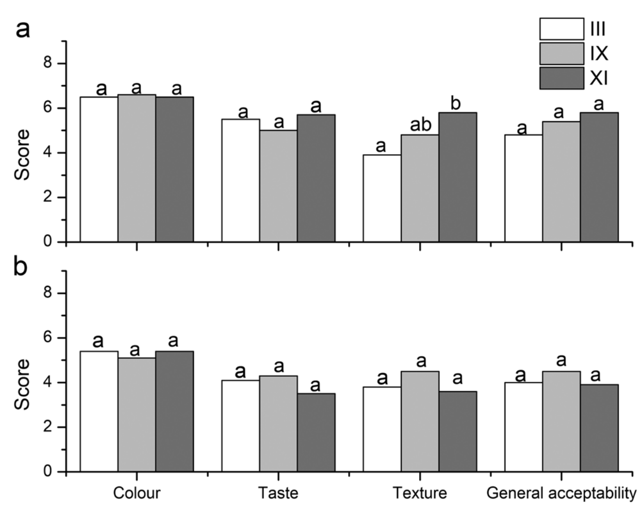

Figure 5: Scores of the colour, taste, texture, and general acceptability obtained in the sensory analysis of (a) biscuits prepared with formulations III, IX, or XI with sugar and (b) biscuits prepared with formulations III and IX with sugar and algarrobo flour (s + AF).

5-score value is within the evaluated ranges for all samples, it can be concluded that the biscuits were acceptable (none were rejected). Other authors found slightly higher acceptability scores in algarroba muffins [11] and “panettone-like” bread [10] (scores 6 and 6.2, respectively, in hedonic scale with untrained panellists). However, these authors used AF as flour replacement, and they used different food systems. In the present work, AF was used as a partial sugar replacement in biscuits. This is a much difficult task because the presence of sugar affects not only the flavour, but also the dimensions, texture, surface colour of the biscuits, and its replacement has a much direct effect on the general acceptability of this foodstuff [4,39].

4 Conclusion

In the present work AF was obtained from *Prosopis chilensis* pods and its proximate composition was determined (9.81% proteins; 4.58% lipids; 7.18% moisture; 4.50% ash; and 73.93% carbohydrates) as well as its amino acid profile. Results showed that glutamic acid, aspartic acid, and isoleucine were the most abundant amino acids in AF. A total of 11 formulations of gluten-free biscuits with AF were prepared with different proportions of RF, CF, MS, and CS, and their physical characteristics were analysed. Results showed that the colour of the biscuits is related to the colour of the ingredients used in the formulations and also to the extent of Maillard reactions.

Three selected dough formulations were made, and the effect of the AF as a partial sugar replacer was evaluated. These formulations were firmer and less adhesive than the dough prepared with sugar only. The antioxidant activity and the total fibre increased in samples with AF (probably because of the presence of phenolic components), while no differences were found in the size, weight, moisture, or texture of the biscuits. The sensory analysis indicated that the developed products would be acceptable for consumers.

It could be concluded that the AF could be used as a gluten-free ingredient that increases the antioxidant activity and the fibre content of the biscuit formulations, without significantly changing the biscuit texture.

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Data availability statement: The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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