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Formulation and Sensory Evaluation of *Prosopis alba* (Algarrobo) Pulp Cookies with Increased Iron and Calcium Dialyzabilities

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Abstract. *Prosopis alba* (algarrobo) is an important indigenous specie, which fruits are used as food and feed since ancient times. Cookies containing algarrobo pulp (AP) with increased iron and calcium availabilities were formulated and sensory evaluated. AP is preferred as food ingredient because of its high sugar content and pleasant flavour. Formulated cookies mean proximal composition was 8.9 g/100 g protein, 7.2 g/100 g dietary fiber, 25 g/100 g total sugar, and 18.5 g/100 g crude fat with iron and calcium contents 30 ppm and 340 ppm, respectively. Ascorbic (AA) and citric (CA) acids at different mM acid: mM Fe were added in order to increase mineral availabilities being evaluated by an *in vitro* method. Those ratios were 5:1 and 10:1 for AA:Fe whereas for CA:Fe were 50:1 and 100:1 and combinations of them. After chosen the best AA:Fe and CA:Fe ratios (5:1 and 50:1, respectively), sensory evaluation with trained sensory panel and a consumer acceptability test with one hundred and seventy untrained judges were carried out. Acceptability test showed that 77.65% of the people (<25 years old 41.76%, 25–50 years old 20.00% and>50 years old 15.89%) tasting final formulated cookies indicated that they "like very much" or "moderately like" and there were not consumers rejecting them.

Key words: *Prosopis*, *Prosopis* cookies, Iron availability enhancers, Calcium availability enhancers, Iron dialyzability

Introduction

Algarrobo (*Prosopis alba*) is an indigenous Leguminous tree named like that by Spanish conquerors because of its similarity with the European algarrobo (*Ceratonia siliqua*). This specie is widespread in Argentina and other regions of America being like other *Prosopis*, important because of its use as food, feed and fuel material and its resistance to drought and saline soils [1].

Its fruits, which are indehiscent pods, can be separated into exocarp, mesocarp (pulp), endocarp and seeds. The algarrobo pulp (AP) is used to prepare different foods, being appreciated because of their high sugar content and pleasant flavour. In a previous work, it was found not only an interesting calcium and iron levels in AP but important dietary fiber contents [2].

Ascorbic acid (AA) is widely accepted as an efficient non heme iron availability enhancer helping to maintain iron as its reduced form, which because of its solubility, is more bioavailable in the gastrointestinal tract [2–5]. Also citric (CA) and tartaric acids have a beneficial effect because of its ability to form soluble complexes in the lumen of the small intestine not only with iron but with calcium as well [3, 6]. Regarding calcium availability, some works concluded that not only citrate but also malate, lactate and mixes of citrate-malate acids could enhance calcium availability from foods [7].

As calcium and iron deficiencies are commonly recognized as nutritional disorders among developing communities, it could be interesting to evaluate how to increase mineral availability from food with indigenous materials as ingredients.

The objective of this work was to develop a cookie formulation containing algarrobo pulp with increased iron and calcium availabilities and good acceptability. Ascorbic and/or citric acids were added to cookie formulation, at different levels, based on previous work with *Prosopis ruscifolia* pulp which demonstrated that those organic acids could enhance endogenous iron dialyzability [2]. In order to evaluate its nutritional importance, not only iron and calcium but also polyphenols dialyzabilities were determined in AP cookies. After chosen the best AA and CA levels, sensory evaluation with trained sensory panel and a consumer acceptability test with one hundred and seventy untrained judges were carried out.

Materials and Methods

Materials

AP *Samples*. *Prosopis alba* pods were collected in Chaco province, Argentina, packed in bags and transported to the laboratory. The pods were dried at 50 °C for 72 h. in a convection electric oven (Bioelec, Santa Fe, Argentina), to 4% moisture before milling. Milling was carried out in a disc mill (Pulverizer type, Bico Inc., CA) obtaining a mixture of pods fractions from which the pulp was separated using a 0.42 mm square hole screen.

Cookie Preparation. In previous experiments different algarrobo pulp additions were tried in a typical cookie formulation modified by the authors and it was concluded that the maximum level to obtained an acceptable product was the one included in the following recipe [8]. For each cookie

Table 1. Fe:AA and/or Fe:CA molar ratios added to cookies

Samples	Fe:AA[a]	Fe:CA[b]
Control	–	–
1	1:5	–
2	–	1:50
3	1:5	1:50
4	1:10	–
5	–	1:100
6	1:10	1:100

[a]Fe: AA = Fe:ascorbic acid molar ratio.
[b]Fe: CA = Fe:citric acid molar ratio.

sample preparation; 70 g wheat flour, 30 g algarrobo pulp, 15 g sugar, 0.6 g NaCl, 25 g shortening, 1 g ammonium bicarbonate and 0.6 g sodium bicarbonate were placed in a bowl and mixed in a pastry blender. Depending on samples, ascorbic and/or citric acids were added with the ingredients at different molar ratios Fe: ascorbic acid (AA): 1:5, 1:10; Fe: citric acid (CA): 1:50, 1:100 or combinations of them (Table 1). After that, 25 ml water was added slowly to make a dough. Then the dough was rolled, formed and cookie dough units were placed on a baking sheet and baked at 190 °C during 5 min.

Cookies were evaluated 24 h. after baking for chemical composition, mineral and polyphenol dialyzabilities and also they were sensory analyzed.

Analytical Methods

Moisture, protein (Nx6.25) (macro Kjeldahl) and crude fat were determined using AOAC methods. Total sugars were evaluated by Lane and Eynon's method. Iron and calcium were evaluated on AP or cookies in a IL-551 atomic spectrophotometer after dry ashing of samples. Dietary fiber was determined by an enzymatic gravimetric procedure [9].

Total polyphenols were determined by Folin Denis method expressing results as mg tannic acid Eq/100 g [10].

Mineral Dialyzability. A modification of the widespread in vitro Miller's method according to Wolfgor et al. was followed [11, 12]. Aliquots (12 g) of homogenized samples prepared to 30% (w/w) were adjusted to pH 2.0 with 6 N HCl and after addition of 0.38 mL pepsin digestion mixture (16% pepsin solution in 0.1 N HCl), were incubated at 37 °C during 2 h in a shaking water bath. At the end of pepsin digestion, dialysis bags containing 15 mL PIPES buffer were placed in each flask. Buffer molarity used for each particular formula was calculated in order to obtain a final pH of digest-dialyzate 6.5 ± 0.2. Main factors taken into account to calculate buffer molarity were: (1) HCl mEq needed to reach pH 2; (2) HCl mEq incorporated with pepsin solution; (3) intrinsic acidity mEq [11]. Since intrinsic pH of food was approximately 7, there was no need to consider it, except for cookies added with citric acid; (4) mEq generated by hydrolysis, which were calculated as follows: each pepsin digest plus bile-pancreatin solution was adjusted to pH 6.5 and incubated during 120 min at 37 °C. Acid mEq generated by hydrolysis were calculated by subsequent titration to pH 6.5 with 0.1 N NaOH. For all these samples mEq generated by hydrolysis resulted insignificant.

To calculate the buffer molarity the following equations were utilized:

$$M = \left(\frac{\text{Total Acid mEq} + f \times \text{Total Acid mEq}}{fV} \right)$$

where $f = [10^{(\text{PIPES pKa} - \text{desired final pH})}] = 10^{(6.8-6.5)} = 1.995$ V = volume of buffer in dialysis bag. Total Acid mEq resulted from adding: (a) HCl mEq needed to adjust each food matrix to pH 2; (b) HCl mEq from pepsin solution in the aliquot of pepsin digest (0.038); and (c) intrinsic acidity mEq (mEq to reach pH 6.5 in samples with pH lower than 6.5);

Samples were incubated for 50 min in a shaking water bath at 37 °C. Pancreatin-bile mixture (3 mL of 2.5% bile, 0.4% pancreatin solution in 0.1 N $NaHCO_3$) was then added to each flask and the incubation continued for another 2 h. At the end of pancreatin-bile incubation, bag contents were transferred to weighed flasks, weighed and analyzed for its mineral content by flame atomic absorption spectroscopy (AAS). Assessment of minerals was made by AAS after dry ashing. Lanthanum was added to a 0.2% final concentration to all samples and standards analyzed for Ca in order to correct for possible phosphate interference.

Mineral dialyzability was calculated from the amount of each dialyzed mineral expressed as a percentage of the total amount present in each sample.

$$\text{Dialyzable Mineral}(\%) = \left[\frac{D}{W \times A} \right] 100$$

where D is the total amount of dialyzed mineral (μg); W is the weight of sample (g) and A is the concentration of each mineral in the sample (μg/g).

Dialyzability was performed 24 h after cookies elaboration. Each experiment was carried out at least twice and all analyses were performed in duplicate. Average values were reported.

Trained Sensory Panel. A descriptive sensory evaluation was conducted for aromatics, surface colour, surface appearance, fragility, air cells, astringency, sweet taste, genuine flavour and residual sensation. Seven panelists (three males and four females) with experience in sensory evaluation were selected from the Instituto de Tecnología de Alimentos (Facultad de Ingeniería Química, Universidad Nacional del Litoral, Argentina) [13]. They were all trained in recognition of the selected attributes, in the use of scales

Table 2. Cookie descriptors and definitions

Descriptor	Definition	Limits	
		Lower (1)	Upper (9)
Aromatics	Aromatics associated with roasted cocoa beans and vanilla	Weak	Strong
Surface colour	Associated with dark chocolate	Light	Dark
Surface appearance	Homogeneous colour without dark dots	Very dotted	Non dotted
Fragility	The easiness with which cookies can be broken in two parts	Crumbly	Rigid
Air cells	Shape, size and regular distribution of air cells in cookies	Non uniform	Uniform
Astringency	The chemical feeling factor on the tongue described as pungering/dry and associated with strong tea	Weak	Strong
Sweet taste	The taste on the tongue associated with sugars	Weak	Strong
Genuine flavour	The flavor notes derived from honey, vanilla and chocolate	Weak	Strong
Residual sensation	Any texture or flavour characteristic that is felt after eating the cookie	Weak	Strong

and in the development and use of the descriptors. The panel training, during which judges evaluated the standard cookies and attributes, was carried out during 12 h (six 2-h sessions) [14].

Cookie descriptors and definitions are listed in Table 2.

A 9-point unstructured descriptive scale was used to rate sensory evaluations attributes. The line scale was 100 mm long, anchored 10 mm from either ends corresponding to the lower and upper limits, respectively.

Panel sessions were conducted under fluorescent light in a special room provided with individual sensory booths. The study consisted of six sessions in which two samples were presented in a random order to the panelists, each sample being evaluated four times. Additionally, each panelist received spit cups and a score card. The score cards included a line scale for each attribute. Panelists scored the perceived intensity of each attribute by placing a vertical line across the unstructured scale line. Quantitation was accomplished by measuring the distance from the left end (0.00) to the vertical line, reporting measurements in cm.

Also, panelists were instructed to smell the samples initially to evaluate aromatics and then descriptors such as texture and flavor. Crackers without salt and distilled water were used to rinse the palate between samples.

Consumer Panel. Based on trained sensory panel results, the best cookie formulation was tried in the consumer panel. One hundred and seventy consumers were recruited among people from Santa Fe, Argentina, males and females, with ages ranging from 20 to 60 yrs. They completed a questionnaire asking about their frequency of consumption of any kind of cookies and those who stated consuming them at least once a week were chosen for the present study [15].

Each consumer received three cookies of the same formulation, scoring them using a scale with nine boxes with the extremes labeled "dislike very much" and "like very much". Cookies formulation was that of the one considered the best in trained sensory panel.

Statistical Analysis. Means and standard deviations were calculated for analysis of chemical composition and mineral dialyzabilities.

One way analysis of variance was performed on mineral dialyzability results and trained sensory panel data and the means were compared according to Duncan's multiple range test. When a significant F-value was obtained from means analysis, least square mean differences were examined using conservative $p < 0.05$ values for mean comparison. [16].

On the consumer data, percentual frequencies were established.

Results and Discussion

Analysis of AP, ingredient for cookie preparation, showed following mean and standard deviation values 9.7 ± 0.43 g/100g protein, 21 ± 1.2 g/100 g dietary fiber, 52 ± 2.5 g/100 g total sugar, 5.5 ± 0.27 g/100 g crude fat, 105 ± 3.8 ppm Fe and 980 ± 28 ppm Ca. Those parameters and also crude fat were evaluated in cookies obtaining: 8.9 ± 0.45 g/100 g protein, 7.2 ± 0.44 g/100 g dietary fiber, 25 g $\pm$ 2.9/100 g total sugar, 18.5 ± 1.1 g/100 g crude fat, 30 ± 2.5 ppm Fe and 340 ± 13 ppmCa.

Regarding total phenolics analysis, the results were 890 ± 50 mg tannic acid Eq/100g for AP whereas in cookies 265 ± 36 mg tannic acid Eq/100g were found, there were no statistical differences between the six cookies samples evaluated ($p < 0.05$) Taking into account total phenolics in AP and its proportion in basic cookie formulation, these results showed no important phenolics looses due to cooking process as it was found in other foods [17].

Mineral Dialyzability. In order to estimate iron and calcium availabilities, an in vitro technique was used determining mineral dialyzabilities after a simulated gastrointestinal digestion. This type of methodology is considered useful to

determine the effect of many inhibitors/enhancing dietary factors not only for iron but for calcium availability [7, 18].

The major determinant of Fe bioavailability is the proportion of the nutrient that is absorbed from the gastrointestinal tract which is greatly influenced by physicochemical and dietary factors in the lumen. Regarding calcium, absorption is controlled by complex homeostatic mechanisms and many dietary factors can influence not only calcium absorption but urinary calcium excretion and retention. This makes more difficult to use any in vitro availability results as an estimation of calcium bioavailability. However, aside mechanism involved in calcium homeostasis, soluble calcium in the gastrointestinal tract is needed for its absorption and calcium dializability results could give useful information [7, 18].

Algarrobo pulp mineral dialyzabilities were: 2.4 ± 0.6 for DFe%, 25.0 ± 0.5 for DCa% and 26.36 ± 0.8 for DZn%.

AP cookies showed less iron (DFe%) and calcium availability (DCa%) (Nd for DFe% and 13.5 for DCa%) than those of AP. It could be due to some interactions between ingredients and iron and calcium insoluble forms generated during baking [19].

AP cookies iron and calcium dialyzabilities are shown in Table 3. Zinc dialyzability (DZn%) was the same for all samples (28.6 ± 0.7).

Both levels of CA (1:50 and 1:100) favoured DFe% in the same extension than AA in 1:5 and 1:10 Fe: AA molar ratio whereas DCa% was favoured only by CA additions (Table 3). Once again AA proved to be a potent enhancer of iron absorption [2, 5, 6]. CA has a beneficial effect on not only DFe% but DCa% owing to its ability to form soluble complexes with both minerals [2, 5, 6].

The simultaneous additions of AA and CA resulted in a better DFe% than the one obtained when AA or CA were added alone (Table 3). This is in agreement with Suzuki et al. and Bernardi et al., who found an enhancement of DFe% but no synergistic effect when AA and CA were added together [2, 20].

Table 3. Iron and calcium dialyzabilities in cookies

Samples	DFe%	DCa%
Control	nd	13.5 ± 0.2^{a}
(1) Fe:$AA_{1:5}$	2.6 ± 0.01^{a}	13.1 ± 0.03^{a}
(2) Fe:$CA_{1:50}$	2.6 ± 0.3^{a}	27.0 ± 0.5^{b}
(3) Fe:$AA_{1:5}$ Fe:$CA_{1:50}$	4.0 ± 0.01^{b}	26.6 ± 0.8^{b}
(4) Fe:$AA_{1:10}$	2.1 ± 0.2^{a}	13.1 ± 0.5^{a}
(5) Fe:$CA_{1:100}$	2.3 ± 0.1^{a}	36.0 ± 0.2^{c}
(6) Fe:$AA_{1:10}$ Fe:$CA_{1:100}$	4.8 ± 0.6^{c}	35.6 ± 0.8^{c}

Note. Nd: non detectable iron in the dialyzate. Different letters mean significant differences ($p < 0.05$) AA: Ascorbic acid. CA: Citric acid.

Ascorbic acid is a known enhancer of iron bioavailability, its acts reducing ferric iron and forming soluble complexes with ferrous and ferric species. Because AA forms soluble complexes with food iron at lower pH than inhibitors ligands, iron solubilization is favoured at the acid environment of the stomach and at the neutral environment of the duodenum, promoting its absorption [21–24]. Samples 1 and 4, containing 1:5 and 1:10 Fe:AA molar ratios, showed an important DFe% increase respect to the control. This is in agreement with Siegenberg et al. who showed that AA counteracts inhibitory effects of polyphenols and phytic acid [21]. Related to iron availability inhibitors in *Prosopis* pulp, it is interesting to point out that phytic acid content is negligible but polyphenols content is considerable [2].

Citric acid addition proved to be a good iron and calcium availabilities enhancer at levels used in cookie formulations, as it is shown in samples containing 1:50 and 1:100 Fe:CA molar ratios in Table 3. These results are according to previous studies where CA showed to be an enhancing factor, being DCa% cookies similar to milk and nuts calcium bioavailabilities which are 32% and 21% respectively [7, 25].

Polyphenols Dialyzability. The beneficial effects of absorbed flavonoids in a variety of human disorders are well known. They influence many biological functions including protein synthesis, cell proliferation differentiation and angiogenesis, so it would be interesting to evaluate its availability in cookies [26].

Total polyphenols, evaluated in dialyzates by Folin Denis method, resulted in $30 \pm 5\%$ dialyzability showing no statistical difference among samples. An in vitro methodology was used by other researchers to evaluate citrus flavonoids availability by Miller's method and they found that results could be comparable with those obtained by an *in vivo* methodology [27]. It is interesting to point out that research conducted in last years has shown conclusively that some flavonoids are absorbed with relatively high efficiency, evaluating them by *in vivo* methods [28].

Trained Sensory Panel. All cookies formulations presented in Table 1 were tested in a preliminary session where trained panelists decided that cookies with Fe:CA equal to 1:100 would be not subsequently evaluated because of its tartness derived from the high citric acid contents. DFe% from samples with Fe: AA equal to 1:5 was not different from the one containing 1:10 so the last one was not evaluated. After that, sample with Fe:AA and Fe:CA molar ratios equal to 1:5 and 1:50, respectively (sample 3), having an interesting DFe% and DCa% was chosen and also samples containing 1:5 Fe:AA (sample 1) and 1:50 Fe:CA (sample 2) in order to evaluate if AA or CA contributed to sensory acceptability of trained panelists.

Table 4. Mean score ratings of attributes evaluated in cookie samples

	Samples		
Attributes	1	2	3
Aromatics	4.38^{a}	4.65^{a}	5.11^{a}
Surface colour	6.60^{c}	4.59^{b}	3.76^{a}
Surface appearance	7.07^{a}	6.81^{a}	7.01^{a}
Fragility	4.84^{a}	4.80^{a}	5.20^{a}
Air cells	5.90^{c}	4.85^{b}	3.54^{a}
Astringency	2.50^{a}	2.67^{a}	2.23^{a}
Sweet taste	4.29^{a}	3.66^{a}	3.82^{a}
Genuine flavor	4.58^{b}	3.76^{a}	4.15^{ab}
Residual sensation	1.31^{a}	1.30^{a}	1.24^{a}

Note. Different letters mean significant differences at level $p < 0.05$.

Mean score ratings of evaluated attributes are shown in Table 4. Surface appearance from not only sample 1 but also 2 and 3 showed to be good (6.81–7.07 intensity scale) in trained sensory evaluation. Also mentioned samples had moderate aromatics (4.38–5.11 intensity scale), fragility (4.8–5.2 intensity scale), sweet taste (3.66–4.29 intensity scale),and also they had weak astringency (2.23–2.67 intensity scale) and residual sensation (1.23–1.31 intensity scale). There was not found any statistical difference for the descriptors mentioned above (Table 4).

Surface colour was intense in sample 1 (score 6.6), this sample did not contain citric acid. It is important to consider that, in this condition, polyphenol oxidation and Maillard reactions are favoured because of a higher pH. There were significant differences between the samples ($p < 0.05$).

About air cells, sample 1 was more uniform than the others and the differences were significant ($p < 0.05$). Samples 2 and 3 contained citric acid which contributes, reacting sodium and ammonium bicarbonates present in cookie formulation, to generate more gas bubbles during mixing and baking.

In respect of genuine flavour, the score was moderate and it was found significant differences between samples 1 and 2.

Regarding sample 1 (containing only AA), it could be observed that not only a better colour but an improved air cells scoring was obtained. Other attributes were similar for the tested samples.

As sensory evaluation differences among the evaluated samples were not considered important, it was concluded that sample 3, the one with higher iron and calcium availabilities, would like consumers and with this formulation a consumer acceptability panel was carried out.

Consumer Panel. The percentual frequencies calculated from consumer acceptability results showed that 77.65% of the people (<25 years old 41,76%, 25–50 years old 20,00% and >50 years old 15,89%) tasting sample 3 indicated that they "like very much" or "moderately like" and there were not consumers rejecting it (Fig. 1).

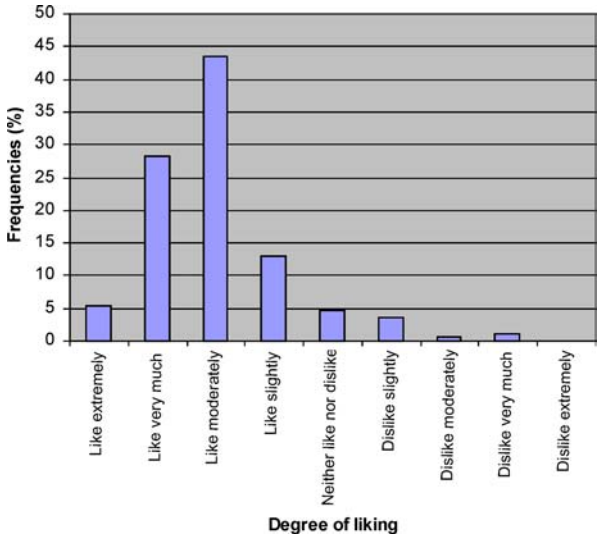

Figure 1. Results from acceptability test, frequencies (%) vs. degree of liking ($n = 170$).

Conclusion

It is concluded that using an indigenous ingredient such as algarrobo pulp, used as such since ancient times, is possible to prepare cookies with good acceptability among consumers. The formulated cookies could contribute to increase mineral and dietary fiber intake in undeveloped communities as a one hundred grams portion of them supplies 17% Fe RDI (Reference Daily Intake), nearly 4% Ca RDI and 30% dietary fiber RDI. DFe% and DCa% in cookies were increased from non detectable and 13%, without ascorbic and citric acids additions, to 4% and 26.6%, when those acids were added. Besides, it is important to keep in mind the ecological importance of *Prosopis alba* being resistant to drought and saline soils and so the value of finding novel uses for its fruits.

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