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Unexpected flavors: development of crackers with nonconventional food plants as calcium source using experimental design

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Abstract

Alternative calcium sources, such as nonconventional food plants, are essential for those who avoid dairy. However, consuming plant-based calcium with meals may compete for iron absorption. The aim of this study was to identify potential calcium sources among these plants and develop a plant-based cracker. Amaranthus viridis, Anredera cordifolia, Lactuca canadensis, Pereskia aculeata, Portulaca oleracea, and Stachys byzantine were studied. After flour was produced, proximate composition and calcium and phytate content were determined. The cracker formulations were defined using the Plackett–Burman design, followed by the Central Composite Rotatable Design, and the acceptance score was the response variable ($p \leq 1\%$). All samples had high calcium content, above 1,000 mg.100 g^{-1} of flour. Considering the phytate:calcium ratio, P. aculeata was selected for cracker preparation. In the first design, the significant variables were vegetable flour and coconut oil. These ingredients varied in the second design, in which the proposed model was significant but did not have a satisfactory R-value (12.60%), probably due to the lack of discrimination between the samples. However, in the second design, the average scores increased (first = 4.1 and second = 5.6), indicating that acceptance was better. In conclusion, P. aculeata is a potential calcium source and can be incorporated into other preparations, beyond traditional salads.

Keywords: plant-based calcium; new product development; alternative calcium source.

Practical Application: Plants can offer bioavailable calcium and can be incorporated into crackers.

1 INTRODUCTION

Alternative sources of calcium, such as plant-based foods, are important because they meet the needs of various population groups who choose not to consume, cannot consume, or have limited access to dairy products, such as vegetarians, people with cow's milk protein allergy (CMPA) or lactose intolerance, and low-income individuals. Furthermore, recent research indicates that plant-based diets may be an interesting alternative for those seeking a healthy diet, as they are associated with a lower incidence of noncommunicable chronic diseases (Miles et al., 2022; Neuhouser, 2018; Satija & Hu, 2018; Schepers & Annemans, 2018).

One potential alternative source of calcium is dark green leafy vegetables. These foods have high calcium content (Melse-Boonstra, 2020; Słupski et al., 2014), and although they may have lower bioavailability compared to milk and dairy products, values ranging from 71 to 86% compared to milk have been reported depending on the phytate and oxalate content (Charoenkiatkul et al., 2008; Weaver et al., 1999). Among these vegetables, a source that has shown promise is nonconventional food plants (NCFP). However, these plants are typically used in dishes served at lunch and dinner, which may lead to competition in iron absorption, as iron-rich foods are traditionally consumed during these meals. Therefore, alternative ways to consume calcium-rich vegetables as snacks are desirable, similar to the consumption of dairy products.

In this context, the present study aimed to determine which NCFP could be considered a good source of calcium and to develop a cracker with the plant incorporated in its formulation, resulting in a calcium-enriched product of food origin rather than a product of simple additive addition. The study aimed to develop a preparation with natural foods that meet nutritional and sensory demands.

2 MATERIALS AND METHODS

The selection of the NCFP used in the study was based on an analysis of the literature and the availability of local producers at the time of harvest: *Amaranthus viridis*, *Anredera cordifolia*, *Lactuca canadensis*, *Pereskia aculeata*, *Portulaca oleracea*, and *Stachys byzantine*.

2.1 Flour production from the NCFP

The fresh leaves were cleaned in running water, air-dried on paper towels, and subsequently dehydrated in a forced-air oven (Solab, SL-102, Piracicaba, Brazil) at 70°C for approximately 70 h.

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After drying, the leaves were crushed and sieved. The obtained flours were packed in plastic bags and stored in a desiccator until analysis and use for cracker preparation.

2.2 Proximate composition and calcium content determination

The moisture analysis was performed by the gravimetric method, in an oven (Nova Ética, Vargem Grande Paulista, Brazil) at 105°C for approximately 3 h (Instituto Adolfo Lutz, 2008). The fixed mineral residue was determined by incineration in a muffle furnace (Jung, 0712, Blumenau, Brazil) at 550°C (AOAC, 2019). The protein content was obtained by determining total nitrogen, using the Kjeldahl method (AOAC, 2019). The ether extract was obtained by the Soxhlet method (Instituto Adolfo Lutz, 2008). For calcium determination, sample preparation was performed by calcination in a muffle furnace, followed by dilution in 2% nitric acid solution and subsequent analysis by flame atomic absorption spectrometry (Thermo Scientific, model Solaar M5, Waltham, USA) (Gomes, 1996). The flour analyses were performed in triplicate, and the results were expressed on a wet basis.

2.3 Phytate content determination

The methodology described by Haug and Lantzsch (1983) was used, with absorbance read at 517 nm on a spectrophotometer (Thermo Scientific, Evolution 60S, Madison, USA), and the results expressed as absorbance value. Phytate analysis was performed to discriminate which sample would have a better phytate:calcium ratio. To calculate this ratio, as the data had different units of measurement, the calcium and phytate content values were normalized and then used to determine the ratio.

2.4 Cracker development

This step was conducted at the Dietetic Technique Laboratory of the Nutrition Department of the Universidade Federal de Juiz de Fora. A base recipe was selected considering the absence of animal-origin ingredients, the taste, and the possibility of incorporating plant flour. The list of ingredients was chickpea flour (240 g), yellow corn flour (120 g), coconut oil (80 g), chia seed (18 g), garlic powder (5 g), salt (4 g), turmeric (2 g), oregano (2 g), and water (120 mL).

The test formulations were defined using the Plackett–Burman design, which simultaneously assess the effect of various factors, optimizing time and resources in the development. From these data, significant variables were defined, and a new design was performed to optimize the formulation. The Central Composite Rotatable Design (CCRD) was used for this purpose (Rodrigues & Iemma, 2007). The formulations were evaluated by the sensory test described below, with the acceptance score being the response variable in the designs.

2.5 Sensory analysis

To assess the acceptance of these preparations, sensory tests were conducted at the Dietetic Technique Laboratory of the Nutrition Department at the Universidade Federal de Juiz de Fora.

The panelists who participated in the analyses were recruited through posters, social media, and university websites, and participation was voluntary. An acceptance test was conducted using a 9-point hedonic scale with untrained panelists. This test is an affective method, in which the panelist indicates how much they liked or disliked a sample on a scale ranging from Disliked extremely (1) to Liked extremely (9) (Stone & Sidel, 2004). The 9-point scale is most suitable as it provides good discrimination of results, generating stable data under different experimental conditions. Additionally, the intervals between neighboring categories are equal throughout the scale, allowing for the use of various statistical tests for result analysis (Moskowitz & Sidel, 1971). Thus, this method can systematically, and with statistical significance, generate results related to sample acceptance. In addition to the scale, participants were asked about their age and whether they were vegetarian.

In the acceptance tests, participants were informed in advance about the possible allergens present, and those with atopy to any ingredient used were excluded. People of both genders, aged 18 years and above, participated in the sensory analyses. All participants in the sensory tests signed an Informed Consent Form, and this research was evaluated and approved by the Research Ethics Committee of the Universidade Federal de Juiz de Fora (Number 5.521.308).

2.6 Statistical analysis

Quantitative variables were presented as mean and standard deviation. The Plackett–Burman and CCRD designs were performed using the Protimiza Experiment Design software (http://experimental-design.protimiza.com.br). The significance of the models was evaluated by ANOVA. The Protimiza Experiment Design software (http://experimental-design.protimiza.com.br) was also used to plot the graphs. The significance level was set at 1%.

3 RESULTS

3.1 Physicochemical composition

Drying in the oven resulted in flours with low moisture content, with values below 1% (0.29 ± 0.21 to 0.99 ± 0.23 g.100 g^{-1}). The carbohydrate content among the samples was similar, ranging from 56 to 70%, with the exception of *A.viridis*, which had a much lower content (31.30 ± 1.14 g.100 g^{-1}). In contrast, this plant had the highest protein content (46.21 ± 1.23 g.100 g^{-1}), followed by *Stachys byzantina* (31.50 ± 1.17 g.100 g^{-1}). The sample with the highest lipid content in the flour was *Lactuca canadensis* (7.10 ± 0.10 g.100 g^{-1}), but it can be observed that these amounts are low in this and all other plants (Table 1).

All samples had a high calcium content, above 1,000 mg.100 g^{-1} of flour. Considering the phytate:calcium ratio, *P. aculeata* was selected for the cracker preparation (Table 1).

3.2 Sensory analysis

The test samples for the acceptance analysis are described in Table 2 and presented in Figure 1 (10 samples), varying in

Table 1. Physicochemical composition of NCFP flours.

NCFP	Moisture (g.100 g^{-1})	Carbohydrate (g.100 g^{-1})	Protein (g.100 g^{-1})	Lipid (g.100 g^{-1})	Ash (g.100 g^{-1})	Calcium (mg.100 g^{-1})	Phytate (AU)	Phytate: calcium ratio
Amaranthus viridis	0.49 ± 0.09	31.30 ± 1.14	46.21 ± 1.23	4.92 ± 0.16	17.09 ± 0.13	5,115 ± 78	0.86	0.72
Anredera cordifolia	0.53 ± 0.24	63.98 ± 0.62	14.69 ± 1.53	5.59 ± 0.98	15.21 ± 0.08	3,567 ± 69	1.07	1.28
Lactuca canadensis	0.99 ± 0.23	68.88 ± 1.69	12.50 ± 1.63	7.10 ± 0.10	12.37 ± 0.03	4,006 ± 70	1.45	1.56
Pereskia aculeata	0.47 ± 0.30	57.33 ± 2.38	18.47 ± 1.35	4.17 ± 0.34	19.56 ± 1.17	6,188 ± 49	0.65	0.45
Portulaca oleracea	0.29 ± 0.21	70.25 ± 0.45	10.23 ± 0.41	3.78 ± 0.43	15.45 ± 0.32	3,555 ± 71	1.17	1.40
Stachys byzantina	0.83 ± 0.25	56.06 ± 1.16	31.50 ± 1.17	2.71 ± 0.24	8.88 ± 0.28	1,601 ± 375	1.41	3.76

Carried out in triplicate and expressed on a wet basis.

Table 2. Cracker formulations for the sensory analysis defined by the Plackett–Burman design.

Ingredients (g)	1	2	3	4	5	6	7	8	9	10
Chickpea flour	240	240	240	200	240	200	200	200	220	220
Yellow corn flour	80	80	120	120	120	80	120	80	100	100
Coconut oil	100	60	60	100	100	100	60	60	80	80
P. aculeata flour	5	45	45	45	5	45	5	5	25	25
Chia seed	18	18	18	18	18	18	18	18	18	18
Oregano	2	2	2	2	2	2	2	2	2	2
Salt	4	4	4	4	4	4	4	4	4	4
Garlic powder	5	5	5	5	5	5	5	5	5	5
Turmeric	2	2	2	2	2	2	2	2	2	2
Water (mL)	120	120	120	120	120	120	120	120	120	120

Table 3. Variables effects (1% significance level).

Name	Effect	Standard error	T calculated	*p*-value
Average	4.33	0.07	58.47	0.0000
Bending	0.37	2.78	0.13	0.8943
Chickpea flour (x_1)	0.29	0.15	1.98	0.0483
P. aculeata flour (x_2)	1.96	0.15	13.27	0.0000
Yellow Corn flour (x_3)	-0.02	0.15	-0.13	0.8931
Coconut oil (x_4)	-0.49	0.15	-3.32	0.0009

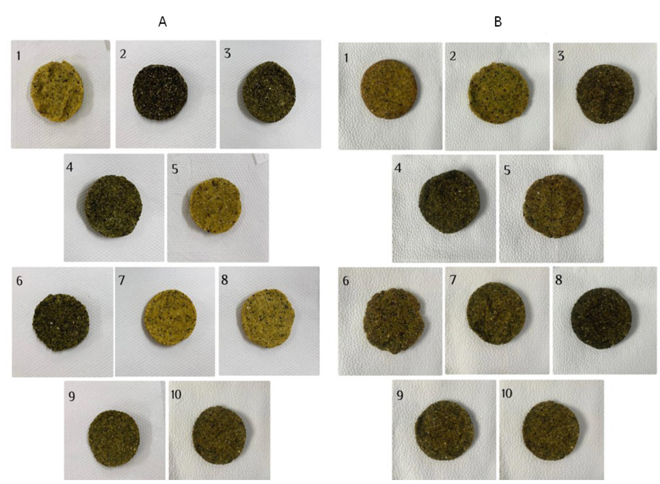

Figure 1. Appearance of the different cracker formulations in the (A) Plackett–Burman design and (B) CCRD.

the quantities of chickpea flour, *P. aculeata* flour, yellow corn flour, and coconut oil.

The sensory tests in the first stage were conducted by 90 untrained panelists, of whom 74.4% were women and 25.6% were men, with an average age of 26.68 ± 10.86 years, and 4.5% were vegetarians.

According to Table 3, the significant variables (1%) in the crackers acceptance were vegetable flour and coconut oil, ingredients that varied in the next design.

In the second design, CCRD, the aim was to define the optimum concentrations of coconut oil and *P. aculeata* flour to obtain the best scores in the overall acceptance evaluation of the crackers. The concentrations of the ingredients can be seen in Table 4, and their appearance is in Figure 1.

After the analysis with 50 untrained panelists, 62.0% women, with an average age of 26.6 ± 10.3 years, and none vegetarian, the proposed model was significant (Table 5) but did not have a satisfactory R-value (12.60%). This may have been due to the lack of discrimination between the different samples, where 8 of the 10 samples had average scores between 5 and 6 (Figure 2).

However, it should be noted that in the second design, the average scores of the samples increased (PB = 4.1 and CCRD = 5.6) (Figure 2), indicating that overall acceptance was better.

Table 4. Cracker formulations defined by CCRD.

Ingredients (g)	1	2	3	4	5	6	7	8	9	10
Coconut oil	60	100	60	100	51.7	108.3	80	80	80	80
P. aculeata flour	10	10	45	45	27.5	27.5	27.5	52.25	27.5	27.5
Chickpea flour	220	220	220	220	220	220	220	220	220	220
Yellow corn flour	100	100	100	100	100	100	100	100	100	100
Chia seed	18	18	18	18	18	18	18	18	18	18
Oregano	2	2	2	2	2	2	2	2	2	2
Salt	4	4	4	4	4	4	4	4	4	4
Garlic powder	5	5	5	5	5	5	5	5	5	5
Turmeric	2	2	2	2	2	2	2	2	2	2
Water (mL)	120	120	120	120	120	120	120	120	120	120

Table 5. Results of ANOVA using DCCR.

Source of variation	Sum of squares	Degrees of freedom	Mean square	Fcalc	*p*-value
Regression	183.9	3	61.3	19.1	0.00000
Residues	1,276.1	398	3.2		
Lack of adjustment	9.3	5	1.9	0.6	0.71617
Pure error	1,266.7	393	3.2		
Total	1,460.0	401			

4 DISCUSSION

Vegetables, such as the NCFP studied, are generally highly perishable once harvested. One reason for this is their high moisture and water activity. Drying the leaves, as proposed, resulted in products with low moisture content, with values below 1%. According to water adsorption isotherm studies, moisture values lower than 10% for rice flour (Abdullah et al., 2000), 13% for amaranth flour (Balderrama & Cadima, 2014), and 14% for five species of African leafy vegetables—*Corchorus olitorius* (jute mallow), *Crotalaria ochroleuca* (slender leaf), *Vigna unguiculata* (cowpea), *Solanum villosum* (nightshade), and *Amaranthus blitum* (amaranthus) (Hag et al., 2020)—are associated with beneficial water activity values for food preservation. The concept of water activity is the most useful expression of water availability for microbial growth (Lacey & Magan, 1991) and enzyme activity (Drapron, 1985). Thus, considering the moisture content found, it can be inferred that the flours would possibly have a lower risk of microbial and enzymatic activity.

The *A. viridis* sample had a high protein content, with values similar to chickpeas (TACO, 2011), a legume considered a source of this macronutrient. It should also be noted that in this study, we found a value higher than that reported in the literature by Silva et al. (2021) (16.49 ± 0.18 g.100 g^{-1} of protein on a dry basis). This difference may have occurred due to the diversity in composition often observed depending on the location and cultivation method of the plants (Vega et al., 2020). Although plant proteins are deficient in essential amino acids, the in vitro digestibility percentage of this macronutrient in *A. viridis* was 90% according to Silva et al. (2021), making it a promising source when combined with other foods.

Also, an analysis conducted in Japan with various species of Amaranthus found that *A. viridis* had the highest protein content among the other species evaluated (29.06 g.100 g^{-1} DW protein), including total amino acids (89.57 g.100 g^{-1} DW protein), predominating glutamine, asparagine, leucine, and alanine. *A. viridis* also presented a good source of lysine (6.24 g.100 g^{-1} DW protein), an essential amino acid limited in cereals. In contrast, cysteine and methionine were present in low quantities, and the consumption of cereals was recommended to compensate for this limitation (Andini et al., 2013).

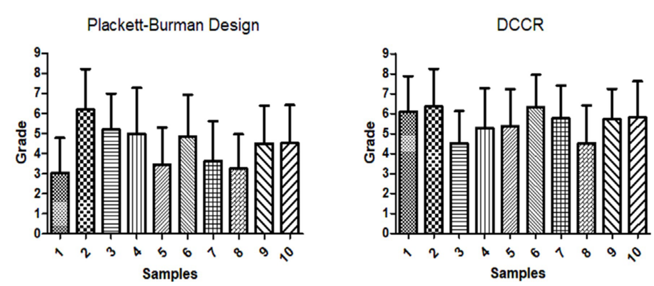

Figure 2. Average scores of the different samples in the two experimental designs.

The plants with the highest calcium content were *A. viridis* and *P. aculeata*, with values higher than those reported in the literature by Silva et al. (2021) (25.85 ± 0.12 g.100 g^{-1} of calcium in dry basis) and Vega et al. (2020) (250.3–476.1 mg.100 g^{-1} in dry basis), respectively. This variation is also expected for calcium, as the location and cultivation method influence the mineral concentration in plants (Bourassa et al., 2022; White & Broadley, 2009).

The calcium content of the evaluated plants exceeds the values found in dairy products, the main sources of animal-derived calcium. For example, whole cow's milk and whole natural yogurt, on average, contain 123 mg and 143 mg of calcium per 100 g of food, respectively (TACO, 2011). Despite this high value, it should be noted that plant-derived calcium usually has lower bioavailability due to the presence of antinutritional factors such as phytate and oxalate. However, there are studies showing that although lower, for some plants like *Coccinia grandis* Voigt. and *Psophocarpus tetragonolobus* [L] DC, bioavailability can be 70–86% compared to dairy values (Charoenkiatkul et al., 2008), and thus, depending on the amount consumed, it is possible to meet calcium requirements using plant sources. By presenting here other possible sources of calcium, in addition to the better-known ones such as spinach, broccoli, and kale (Knez & Stangoulis, 2021), the possibility of meeting calcium requirements using plant sources is increased.

Moreover, the removal of moisture from the plants resulted in flours with higher nutrient contents, such as proteins and calcium, offering a product with higher nutritional density, which can be positive for populations at risk of deficiencies and specific populations such as vegetarians and vegans. It should be noted that some populations often suffer from income constraints, and the ease of cultivation of the plant and thus the possible lower cost favor its use.

Considering the TACO data, where wheat flour and cornmeal have, respectively, 75.1 and 78.9% carbohydrate, 9.8 and 7.2% protein, and 18 mg and 3 mg of calcium per 100 g, replacing a portion of the flours with the NCFP flours is positive, as there would be a higher amount of protein and calcium, and lower carbohydrate.

The biscuit developed in the second design presented 20.0–185.7 mg of calcium per 30 g portion of biscuit (Brasil, 2020), which can contribute from 2.0 to 18.6% of an adult's daily needs (Institute of Medicine, 2011), thus being an alternative source of calcium in the diet.

Furthermore, the presence of bioactive compounds and the potential health benefits (da Silva Porto et al., 2022; de Souza et al., 2022; Silva et al., 2021) reinforce the relevance of the study in attempting to incorporate the plant into a new preparation.

Finally, the scores presented indicate that the formulation developed, incorporating an NCFP with a high calcium content, was not rejected by the testers, as they had average scores above 5, indicating that they were indifferent or slightly liked the cracker developed. Although not yet a very positive result, the evolution in acceptance in both designs proves its validity for improving formulations, indicating that it is possible to incorporate an unconventional ingredient into a cracker.

5 CONCLUSION

Therefore, it is concluded that after the screening of different vegetables' chemical composition, *P. aculeata* stood out in terms of higher calcium and lower phytate contents, being a determining factor for the choice of the plant.

It is possible to affirm that the calcium content present in the dark green leafy vegetables evaluated in this study, the NCFP, especially *P. aculeata* and *A. viridis*, are potential sources of calcium, providing an alternative to complement the nutritional needs of the Brazilian population.

Moreover, it can be concluded that the plants can be incorporated into other preparations, beyond traditional salads and braised preparations, expanding the use of these foods.

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