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Development and characterization of a nut-based beverage of Brazil nut (*Bertholletia excelsa*) and macadamia (*Macadamia integrifolia*)

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Abstract

Brazil nuts and macadamia nuts are highly nutritious and can be used for the production of plant-based beverages. Considering the need to develop new beverages alternative to cow milk, the aim of this study was to develop water-soluble extracts based on Brazil nuts and macadamia nuts using an experimental design for simplex centroid mixtures in order to evaluate the proximate composition of the ternary mixture. The best beverage formulation was established based on sensory acceptance and shelf life, which was determined through microbiological aspects during refrigerated storage. The most accepted formulation consisted of 60% (w/w) of water, 20% (w/w) of Brazil nuts, and 20% (w/w) of macadamia nuts. The nut-based beverage presented a yellow–white color, and it was in accordance with the microbiological standards established by the Brazilian legislation, with an estimated shelf life of 28 days.

Keywords: non-dairy milk; water-soluble extract; lactose free; stability; storage.

Practical application: This new plant-based beverage is an excellent alternative to cow milk, with interesting nutritional value and good stability.

1 INTRODUCTION

Brazil nuts (*Bertholletia excelsa* Bonpl.) are an important non-timber forest product that grows naturally on non-flooded terraces in the Amazon forest. These nuts are in high demand in the international market due to their nutritional attributes (Condori et al., 2019). Macadamia nuts (*Macadamia integrifolia*) belong to the Proteaceae botanical family and are classified as a subtropical fruit tree. Their nuts contain more than 70% fat and 9% protein, and their consumption is associated with the prevention and treatment of cardiovascular diseases (Xia et al., 2022).

In general, nuts are considered a good source of protein, unsaturated fatty acids, fiber, minerals, vitamins, and phytochemicals and an excellent alternative for the development of plant-based products (Yang et al., 2023). One of the main reasons for researching new plant-based products is to offer consumers tasty, nutritious alternatives to conventional animal products. This shift toward plant-based alternatives is driven by the desire to reduce the environmental impact and improve the sustainability of agricultural food production (Nolden & Forde, 2023).

In this way, the demand for plant-based milk alternatives has increased in recent years, especially, nut-based milk alternatives, due to the nutritional and organoleptic characteristics (Huang et al., 2023).

Many studies have focused on beverages made of Brazil nuts (Barbosa et al., 2020; Vasquez et al., 2021); however, there are no studies about their production interactions with macadamia. Therefore, this study proposed to elaborate a new plant-based beverage using different combinations of water, Brazil nuts, and macadamia nuts. This study evaluated the color, proximate composition, and microbiological and sensorial quality in order to determine its shelf life.

2 MATERIALS AND METHODS

2.1 Materials

The Brazil nuts used in this research were raw with the skin partially removed, with some donated by Delta Castanhas do Brasil Ltda (Iporá, GO, Brazil) and some acquired from Naturalista Produtos Naturais Ltda (Goiânia, GO, Brazil), along with the roasted macadamia nuts in their skins. The citric acid, potassium sorbate, and sodium benzoate were supplied by Casa Forte Ltda. (Goiânia, GO), and the stabilizer carboxymethylcellulose (CMC) was donated by Naturofarma Ltda. (Goiânia, GO).

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2.2 Methods

2.2.1 Processing of the plant-based beverage of Brazil nuts and macadamia nuts

The methodology adopted to produce the water-soluble extract based on Brazil nuts and macadamia nuts was developed by (Cardoso et al., 2020). The nuts were sanitized in a 300 ppm chlorine solution and ground in an industrial blender (LSR 25, Siemsen, Brusque, Santa Catarina, Brazil) for 10 min using 70% of water and 15% of each type of nut. The homogenized product was centrifuged (VCC-7000, Vicini, China), and the liquid phase was heated to 85°C. CMC was then added to the heated extract at 0.2% (w/v), and the preservatives citric acid, sodium benzoate, and potassium sorbate were also added in the proportions of 0.05% (w/v), 0.15% (w/v), and 0.10% (w/v), respectively. Afterward, the extract was homogenized for 5 min, filled into glass bottles with crown-type caps, pasteurized at 80°C for 25 min, cooled, and finally stored under refrigeration (4 ± 2°C).

2.2.2 Experimental design

The proportions of the components in the mixture were defined using a simplex centroid mixture design, aiming to evaluate the behavior of the interaction between the components: water (x_1), Brazil nuts (x_2), and macadamia nuts (x_3) (independent variables), as shown in Table 1.

The response variables for the design were the moisture, ash, lipid, carbohydrate, and protein contents. The three-component mixture design proposed in the present study consisted of seven experiments, with minimum restrictions set at 50% (w/w) for water and a minimal proportion of 10% (w/w) for each of the nuts in each formulation.

2.2.3 Proximate composition

The percentages of moisture, ash, and protein were determined according to AOAC (Horwitz, 2010). The lipid content was determined according to the methodology proposed by Bligh and Dyer (1959), and the carbohydrate content was calculated by difference.

2.2.4 Determination of the best formulation of the nut-based beverage

Sensory analyses were performed with 30 untrained panelists, who were randomly recruited from the Universidade Federal de Goiás (UFG), to select the best formulation and evaluate it

Table 1. Simplex centroid design for processing the nut-based beverage.

Formulation	Pseudo-component			Real concentration (% w/w)		
	x'_1	x'_2	x'_3	x_1	x_2	x_3
1	1	0	0	80	10	10
2	0	1	0	50	40	10
3	0	0	1	50	10	40
4	1/2	1/2	0	65	25	10
5	½	0	1/2	65	10	25
6	0	1/2	1/2	50	25	25
7	1/3	1/3	1/3	60	20	20

during storage. The nut-based beverage (approximately 20 mL) was presented in disposable cups coded with three-digit numbers along with a glass of water for mouth rinsing. A nine-point hedonic scale (1 — extremely dislike it and 9 — extremely like it) was used for the evaluation of the attributes (odor, color, taste, and overall acceptance), and a five-point scale (1 — Certainly I would not buy it and 5 — Certainly I would buy it) was used for the purchase intention test.

2.2.5 Physical and physicochemical stability analyses

Color, pH, and titratable acidity were determined every 7 days over a 28-day period in the best formulation. The instrumental color determination was conducted using an electronic colorimeter (Colorquest II, Hunter Associates Laboratory Inc, Reston, Virginia, USA). The pH was determined using a pH meter (Micronal B474, São Paulo, São Paulo, Brazil), following technique no. 981.12 of the AOAC protocol (Horwitz, 2010). The titratable acidity was determined according to the protocol of Sadler and Murphy (2010) with results expressed as a percentage of oleic acid.

2.2.6 Microbiological analyses

For determination of shelf life, every 7 days, 25 ml of the best formulation of the nut-based beverage was added to 225 ml of 0.1% peptone water, homogenized using a stomacher, and decimal dilutions were performed up to 10^{-3} to detect thermotolerant coliforms, coagulase-positive *Staphylococci* and *Salmonella* sp., as established on item 23a of Resolution RDC nº 216 of Brazil (Brasil, 2002). Detection of molds and yeasts was also performed in order to enhance food safety. All microbiological analyses followed APHA protocols (Salfinger & Tortorello, 2013).

2.3 Statistical analyses

The experimental design utilized the Statistica 7.0 software. All analyses were conducted in triplicate. Experimental data are presented as mean ± standard deviation. A probability level of $p \leq 0.05$ was considered significant for all statistical procedures. The means of proximate composition were compared using a t-test, and the means of physical and physicochemical analyses and sensory evaluation were compared using Tukey's test, utilizing the Statistic 7.0 software (Statsoft).

3 RESULTS AND DISCUSSION

3.1 Proximate composition

Table 2 shows the results obtained for the determinations of dry matter, protein, ash, lipids, and carbohydrates for each of the experiments proposed in the experimental design.

The amount of water used in the experiments also showed a significant difference in all the formulations ($p \leq 0.05$), giving a direct relationship with the response variables, such that for the formulation with the highest proportion of water (formulation 1), the response variables showed their lowest values for concentration, except for carbohydrates, which were calculated by difference. On the contrary, the formulations with smaller proportions of water (formulations 2, 3, and 6) showed higher values for the response variables since the lower the moisture content, the higher the concentration of total solids. When the sum of the amounts of Brazil nuts and macadamia nuts was equal to 50% (formulations 2, 3, and 6), the contents of all the responses showed higher values.

All the formulations proposed by the design presented significant differences in the lipid content. The formulation with a greater Brazil nut content (formulation 2) presented higher values for the response values of dry matter, ash, and proteins, whilst the formulation with a higher content of macadamia nuts (formulation 3) showed higher carbohydrate values. This could possibly be justified by the characterization of the raw materials since Brazil nuts have higher ash and protein contents than macadamia nuts (Cardoso et al., 2020).

The data obtained for the mixture design allowed one to test the specific models of this type of design, such as, for example, the linear, quadratic, and χ^2 models. The analysis of variance was applied between the models at the 5% level ($p < 0.05$) to verify the degree of significance of the model. The experimental determination coefficient (R^2) and the fitted determination coefficient (R_A^2), parameters that show the degree of variability of the model, were also verified in order to choose the best fit. The closer the determination coefficients are to unity, the better the fit to the data. Table 3 shows the results obtained for the coefficients tested with respect to the response variables.

All the models fitted the data of the response variables except the chi-squared model for the responses of dry matter and carbohydrates, for which the p values were not significant ($p < 0.05$). R^2 and R_A^2 showed values close to unity for all the models except for the linear model with respect to carbohydrates, confirming the good fit and good predictive capacity of the models.

Table 4 shows the predictive equations generated to describe the behavior of the variables: dry matter, lipid, ash, protein, and carbohydrate. These models were expressed as a function of the concentrations of water (x_1), Brazil nuts (x_2), and macadamia nuts (x_3) in pseudo-components, with nonsignificant coefficients being eliminated ($p < 0.05$).

Despite the good fit of the models, the quadratic model provided a better fit for the data on dry matter and carbohydrates, whereas the cubic model more accurately represented the lipid, ash, and protein contents of the proposed mixtures. It can be seen from the predictive equations that the coefficients analyzed individually produced a synergistic effect for all the response variables analyzed, contributing to an increase in the contents of the dependent variables (the coefficients of the interactions between x_1, x_2, and x_3 were greater than zero).

Note also that the effect of the action of the mixture coefficients x_1x_2, x_2x_3, and x_1x_3 in binary combinations presented antagonistic effects in the carbohydrate model. In contrast, the interactions of these coefficients led to a significant increase in

Table 2. Physicochemical characterization of the water-soluble extracts based on Brazil nuts (*Bertholletia excelsa*) and macadamia nuts (*Macadamia integrifolia*).

Formulation	Dry matter	Lipid	Ash	Protein	Carbohydrate*
1	13.51^f ± 0.20	8.21^g ± 0.10	0.49^f ± 0.01	2.10^e ± 0.24	2.70^c ± 0.14
2	48.46^a ± 0.80	35.57^c ± 0.03	1.46^a ± 0.01	7.39^a ± 0.24	4.05^b ± 0.75
3	47.72^a ± 0.11	36.38^b ± 0.03	0.87^{de} ± 0.03	4.79^c ± 0.05	5.68^a ± 0.15
4	33.93^d ± 0.23	25.35^f ± 0.07	0.96^c ± 0.02	5.66^b ± 0.38	1.96^c ± 0.57
5	32.55^e ± 0.16	25.64^e ± 0.19	0.80^e ± 0.06	3.71^d ± 0.08	2.39^c ± 0.22
6	46.47^b ± 0.30	36.68^a ± 0.06	1.14^b ± 0.03	5.93^b ± 0.22	2.72^c ± 0.33
7	37.71^c ± 0.01	28.87^d ± 0.11	0.92^{cd} ± 0.01	5.70^b ± 0.15	2.23^c ± 0.04

*Means followed by the same letter in the same column do not differ statistically at the 5% level according to Tukey's test.

Table 3. The p values and experimental (R^2) and fitted (R_A^2) determination coefficients of the models tested for each response variable.

Response variable	Model	p-value	R^2	R_A^2
Dry matter	Linear	0.0001	0.9871	0.9856
	Quadratic	0.0001	0.9993	0.9991
	Special cubic	0.2689	0.9994	0.9991
Lipid	Linear	0.0001	0.9769	0.9743
	Quadratic	0.0001	0.9985	0.9980
	Special cubic	0.0001	0.9999	0.9999
Ash	Linear	0.0001	0.9705	0.9672
	Quadratic	0.0010	0.9846	0.9819
	Special cubic	0.0017	0.9919	0.9898
Protein	Linear	0.0001	0.9234	0.9149
	Quadratic	0.0001	0.9715	0.9664
	Special cubic	0.0041	0.9832	0.9790
Carbohydrate	Linear	0.0091	0.4065	0.3406
	Quadratic	0.0001	0.9223	0.8963
	Special cubic	0.1259	0.9346	0.9066

the protein concentration of the mixed beverage. Analyzing the statistically significant terms of the fitted models, it was shown that the binary combination of water and Brazil nuts (x_1x_2) was significant for all the response variables, except for the ash content.

From the proposed equation for the protein model, it was concluded that the combination between water and macadamia nuts was not significant. However, this same interaction (x_1x_3) presented a synergistic effect for the dry matter, lipid, and ash models and an antagonistic effect only for the carbohydrate model. Considering the predictive equations, all the different combinations could only be proved statistically significant at the 5% level ($p < 0.05$) for the lipid model.

It was shown that the interaction between the three components of the mixture ($x_1x_2x_3$) was not significant for the models proposed for dry matter and carbohydrates, indicating that it did not directly influence the contents of these components. The interaction between the mixture components in a ternary combination ($x_1x_2x_3$) contributed significantly to increasing the protein contents, as evidenced by the high values for this index. In contrast, this ternary mixture presented an antagonistic effect, contributing to a decrease in the lipid and ash contents.

3.2 Determination of the best formulation of the nut-based beverage

All of the seven formulations purposed by the simplex centroid design were evaluated using sensory analyses. Tukey's test was performed at a 5% significance level to verify the differences between the means. The results for the attributes, odor, color, taste, overall acceptance, and purchase intention are presented in Table 5.

The odor, color, and taste attributes had scores between 5.0, "I did not like it nor I disliked it," and 7.0, "I enjoyed moderately," and even though there was a significant difference ($p > 0.05$) between averages, acceptable for nut-based beverages. Formulations 3 and 7 had the highest averages, 6.60 and 6.33, respectively, in the overall impression attribute and did not present a significant difference ($p < 0.05$).

Table 4. Mathematical models for the proximate composition as a function of the contents of water (x_1), Brazil nuts (x_2), and macadamia nuts (x_3) in a mixed water-soluble extract of Brazil nuts (*Bertholletia excelsa*) and macadamia nuts (*Macadamia integrifolia*).

Response variable	Equations
Dry matter	$y = 13.53x_1 + 48.48x_2 + 47.74x_3 + 11.38x_1x_2 + 7.35x_1x_3 - 6.90x_2x_3$
Lipid	$y = 8.21x_1 + 35.57x_2 + 36.38x_3 + 13.85x_1x_2 + 13.39x_1x_3 + 2.80x_2x_3 - 32.15x_1x_2x_3$
Ash	$y = 0.49x_1 + 1.45x_2 + 0.86x_3 + 0.49x_1x_3 - 1.90x_1x_2x_3$
Protein	$y = 2.20x_1 + 7.32x_2 + 4.82x_3 + 3.58x_1x_2 + 13.94x_1x_2x_3$
Carbohydrate	$Y = 2.67x_1 + 4.02x_2 + 5.64x_3 - 5.02x_1x_2 - 6.53x_1x_3 - 7.94x_2x_3$

x_1: the proportion of water in the mixture; x_2: the proportion of Brazil nuts in the mixture; x_3: the proportion of macadamia nuts in the mixture; y: response estimate.

Once formulation 3 is more expensive due to its higher proportion of nuts, formulation 7 was chosen as the most acceptable by the panelists. In addition, formulation 7 contained 60% (w/w) of water, 20% (w/w) of Brazil nuts, and 20% of macadamia and it received almost the same score in the purchase intention test, 3.23, ranging between "Maybe buy it/Maybe not buy it" and "Possibly would buy it." However, the averages of the scores attributed to the purchase intention did not present a statistically significant difference ($p > 0.05$).

There are several mathematical and computational models for determining and optimizing better formulations of food products; however, none of them is capable of replacing human judgment and sensory evaluation of the relevant characteristics of the product (Singh-Ackbarali & Maharaj, 2014). Thus, sensory analysis is still considered an important tool for choosing the best formulation of a product.

3.3 Physical and physicochemical analyses

The mean values for luminosity, chromaticity, and hue angle during 28 days of storage are shown in Table 6. It was observed that the beverage in this study exhibited a significant increase in luminosity and chromaticity parameters, while its hue angle remained practically constant during the storage.

It was observed that during storage there was an increase in the value of luminosity, which varied from 78.53 to 83.94, indicating that the product presented a lighter color, that is, closer to white. The chromaticity, a parameter that indicates the saturation of the color, varied from 7.56 to 10.01. These values indicate a product with a relatively low intensity of coloration since that values in the space of color refer to gray. The hue angle, which provides information about the shade of the color, remained constant during the study, with no significant difference ($p < 0.05$). Hue angle values close to 90 degrees indicate a yellow–white coloration.

The values found for pH and titratable acidity each week are presented in Figure 1. The pH value increased significantly from the first to the second week and then decreased, remaining practically stable until the end of the study. Titratable acidity showed an inverse correlation with pH, with a marked decrease from the first to the second week, and then remained stable until the end of storage ($p < 0.05$).

The values of pH and titratable acidity are important quality parameters for food products. The titratable acidity is related to reactions like decomposition, hydrolysis, oxidation, and fermentation, which produce acidic compounds that, consequently, increase the acidity of the medium (Chim et al., 2013). The decreasing values of titratable acidity in the nut-based beverage are good indicators of shelf life.

3.4 Microbiological analyses

Given the similarity of the present product to "coconut milk," the samples were evaluated according to the standards established by the current Brazilian legislation for "coconut milk." All the evaluated samples complied with the microbiological standards set by the current legislation (RDC nº 12/2001) for "coconut milk": Thermotolerant Coliforms: $< 10^2$ CFU/mL e *Salmonella* sp.

Table 5. Acceptance test and purchase intention for different formulations of nut-based beverage made of Brazil nuts and macadamia.

Formulation	Odor	Color	Taste	Overall impression	Purchase intention
1	6.00^{abc} ± 1.53	6.87^{ab} ± 2.01	5.07^{ab} ± 2.30	5.33^{ab} ± 2.07	3.00^{a} ± 1.14
2	6.57^{ab} ± 1.38	6.53^{ab} ± 1.78	6.03^{ab} ± 1.63	6.30^{ab} ± 1.32	3.40^{a} ± 0.86
3	6.13^{abc} ± 1.57	7.13^{ab} ± 1.57	6.33^{ab} ± 1.79	6.60^{a} ± 1.59	3.30^{a} ± 1.06
4	6.67^{ab} ± 1.52	7.00^{ab} ± 1.34	5.87^{ab} ± 2.05	6.27^{ab} ± 1.70	3.27^{a} ± 1.05
5	5.67^{bc} ± 1.35	6.60^{ab} ± 1.43	4.87^{b} ± 2.00	5.53^{ab} ± 1.43	2.63^{a} ± 0.93
6	5.30^{c} ± 1.24	6.27^{b} ± 1.39	5.30^{ab} ± 2.02	5.73^{ab} ± 1.48	2.97^{a} ± 1.03
7	7.00^{a} ± 1.20	7.20^{ab} ± 1.21	6.27^{ab} ± 1.89	6.33^{ab} ± 1.73	3.23^{a} ± 1.25

Means followed by different letters in the same column differ significantly from each other at a level of 5% p (≤ 0.05) by the t-test.

Table 6. Mean values for luminosity, chromaticity, and hue angle during 28 days of storage.

Day	Luminosity	Chromaticity	Hue angle*
0	81.74^{c} ± 0.20	7.56^{e} ± 0.19	84.00^{ab} ± 0.23
7	78.53^{d} ± 0.32	8.38^{d} ± 0.06	83.15^{b} ± 0.43
14	83.67^{a} ± 0.07	10.01^{a} ± 0.07	83.27^{b} ± 0.18
21	83.94^{a} ± 0.02	9.59^{b} ± 0.06	83.77^{ab} ± 0.10
28	82.67^{b} ± 0.18	9.22^{c} ± 0.12	84.57^{a} ± 0.62

Means followed by different letters in the same column differ significantly from each other at a level of 5% p (≤ 0.05) by Tukey's test; *in degrees.

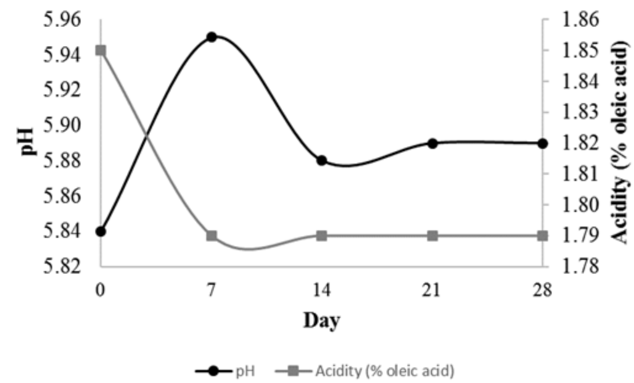

Figure 1. Behavior of pH and titratable acidity of the nut-based beverage made of Brazil nuts and macadamia.

According to Kornacki and Johnson (apud Feng et al., 2020), the presence of coliforms in food is a useful indicator of post-sanitation and post-processing contamination. Since the growth of coliforms was not detected, sanitization processes of Brazil nuts, macadamia, glass bottles, and crown caps, and the pasteurization of the packaged product were satisfactory.

In the same way, it is possible to state, based on the results of microbiological tests, that there was no process of recontamination of the glass bottles and the product during the packaging, as well as, in the period of the evaluation of stability under controlled conditions.

4 CONCLUSION

In general, the mathematical models obtained in this study satisfactorily explained the behaviors of the proximate composition characteristics of the water-soluble extracts based on Brazil nuts and macadamia nuts, presenting high determination coefficients and a nonsignificant lack of fit ($p < 0.05$), demonstrating strong predictive capability. The nut-based beverage made of Brazil nuts and macadamia demonstrated good physicochemical and microbiological stability, allowing us to affirm that the product has a shelf life of 28 days.

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