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Chemical and antioxidant characterization, sensory and shelf-life analysis of cereal bars with almonds from *pequi* (*Caryocar brasiliense* Camb.)

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Abstract

The study aimed to know how almonds from Pequi (*Caryocar brasiliense* Camb.), a byproduct of this fruit from Brazilian Cerrado, can be used to improve value to cereal bars. To respond to this question, the study characterized *pequi* almonds and cereal bars with *pequi*, and evaluate sensory acceptance. The preparation of cereal bars enriched with p*equi* almonds consisted of 3 formulations: standard (0%, p*equi* almond), formulation A (12.5% almond) and formulation B (25.0% almond). The best results were obtained through drying kinetics for the use of almonds in the formulations. Later, they were subjected to physicochemical analyses: moisture, ash and lipids, pH and soluble solids, crude fiber, proteins, sugar content and color index (L*, a* and b*), antioxidant capacity and sensory analysis which relied on 108 consumers. The blind test was applied and the information was used to evaluate the attributes: color, taste, texture, overall impression and purchase intention. *Pequi* almond obtained: 27.99% of fibers, 29.62% of proteins and 35.36% of lipids (composed mainly of oleic acid 57.65%, followed by 27.43% of palmitic acid). The results showed that the sensory acceptance for the consumers increased when they found out that the cereal bars contained *pequi*. Regarding the blind test (without information on the product), it was concluded that consumers appreciate new products with this fruit from the cerrado.

Keywords: Cerrado; color; ABTS; drying.

Practical Application: The potential use of byproducts from Brazilian Cerrado.

1 Introduction

The Brazilian Cerrado is the second largest biome in Brazil and occupies approximately 21% of the territory with a very diversified flora: more than 58 species of native fruits described (Paz et al., 2014). Among these species, *pequi* stands out, not only for its recognized food appreciation, but also for the set of values it represents for populations: economic, cultural, ecological, gastronomic and medicinal (Silva & Tubaldini, 2013). Although *pequi* almond, which lies within the prickly seed, is an oil source with a clear light color and a mild aroma, it is a little known byproduct (Oliveira & Scariot, 2010).

Fruit byproducts seem to be healthy and economically advantageous because they are considered an unconventional nutrient source and due to the increasing consumer awareness regarding healthy eating (Pertuzatti et al., 2020). This has been evidenced by a significant increase in the supply of food with claims of functional properties, among which stand out cereal bars, especially those with nuts, almonds and peanuts.

P*equi is* a widely known fruit which is characteristic of several Brazilian states, but it is still unknown in some regions of the country. In order to popularize the fruit and use its byproduct for creating an opportunity for local growers to gain access to new markets, the present work aimed to study the characterization of *pequi* almonds, their addition in cereal bars and the sensory evaluation and analysis of the cereal bars.

2 Materials and methods

The *pequi* fruits were collected in the district of Araguaiana - Mato Grosso (geographic coordinates: 15° 44' 02" S, 51° 49' 53" W) from adult trees from December 2015 to March 2016. The fruits were stored in plastic bags and refrigerated at 4 °C until further use.

Kinetic drying curves of *pequi* almonds (moisture in function of temperature of 70, 80 and 90 °C) were constructed through tests with almond beds with a height of more than 0.10 m. The almonds were dried in an oven with forced air circulation (Model 400/3 ND, Nova Ética, Brazil) and with Proportional-Integral-Derivative (PID) temperature control (Model SL100/100, SOLAB, Brazil). Almond samples were manually removed from the bed at regular intervals of 600 s and subsequently used for the determination of moisture by conventional gravimetry with a digital balance (precision of $\pm 10^{-4}$ kg) (Model AY220, Marte, Brazil), according to the methodology described by (International Standards Organization, 1980).

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2.1 Elaboration of cereal bars

Three formulations of cereal bars were prepared (Figure 1). The standard formulation had no addition of *pequi* almonds, while formulation A had 12.5% of *pequi* almond and formulation B had 25.0% of *pequi* almond. The other ingredients used in the elaboration of the cereal bars were acquired in local commerce in the city of Barra do Garças - MT: rice flakes - Harald, oat flakes – Natural Life, raisins – La Violetera, dried and crushed *pequi* almonds, ground peanuts - Yoki, honey - Apis Vida, butter - Piracanjuba, vanilla essence - Fleischmann and brown flaxseed – Natural Life (Table 1).

The processing of the formulations of the cereal bars was carried out in a batch type system according to Moraes et al. (2007). To prepare the dry phase, the rice and oat flakes were dried in a conventional oven (Model 402-5D, Nova Ética, Brazil) for five minutes at 180 °C, followed by addition and mixing of *pequi* almonds, raisins, ground peanuts and brown flaxseed. Subsequently, the dry ingredients were mixed to the liquid phase (honey, butter and vanilla essence) under a heating plate (Model NT338, Nova Técnica, Brazil) at 70 °C, during approximately 15 minutes, until adequate mixture was achieved. This mixture was then put in a mould covered with aluminum foil, molded and refrigerated at 4 °C. After 15 minutes, the bars were cut into pieces (3 × 2.5 × 2 cm) packed in aluminum foil and stored at -18 °C until the analyses were performed.

(a) (b) (c)

Figure 1. Cereal bars formulations, (a) formulation a; (b) formulation b; (c) standard formulation.

Table 1. Percentage of ingredients used in the processing of cereal bar formulations.

Ingredients	Formulation A (g/100 g)	Formulation B (g/100 g)	Standard Formulation (g/100 g)
Rice flakes	25.7	25.7	25.7
Oat flakes	24.3	24.3	24.3
Vanilla essence	1.0	1.0	1.0
Raisins	5.0	5.0	5.0
Peanuts	4.0	4.0	4.0
Brown flaxseed	2.0	2.0	2.0
Honey	5.0	5.0	5.0
Butter	3.0	3.0	3.0
Thin oat flakes	5.0	5.0	5.0
Pequi almond	12.5	25.0	-
Water	12.5	-	25

2.2 Physicochemical analysis

The moisture content, ashes and lipids, as well as the determination of pH and soluble solids, were determined according to the methodologies proposed by the Analytical standards of Instituto Adolfo Lutz (1980).

The Association of Official Analytical Chemists guidelines (Association of Official Analytical Chemists, 2002) were used for determining crude fiber, total sugars and protein content based on the nitrogen determination, by using a conversion factor of 6.25.

In order to analyze fatty acids, 100 mg of the sample was weighed. Subsequently, 1 mL of petroleum ether was added and the sample was homogenized. Briefly, 15 mL of hydrochloric acid 0.5 gram-equivalent/L in methyl alcohol was added. The tubes were vortexed and heated at 65 °C for 1 hour, until obtaining a transparent solution. After cooling, 5 mL of isooctane and 6 mL of water were added, and the tubes were homogenized again.

Finally, 1 µL of the upper organic phase was injected into the Perkin-Elmer gas chromatograph. The researchers used an automatic injector and a flame ionization detection (FID) system. During the operation, the detector was maintained at 280 °C and the injector at 250 °C. The injection was performed in split mode (1:50), and the separation was carried out in a DB 23 column (60 m × 0.25 mm x 0.25 µm). The oven was set to 50 °C for 1 min, after which it was heated at 25 °C/min to 175 °C, and then at 4 °C/min to 230 °C. The final temperature was maintained for 20 min. The compounds were identified through retention time of the fatty acids present in the mixture and analyzed under the same conditions. The results were expressed in percentages.

2.3 Antioxidant capacity: capture of free radical ABTS

In order to prepare the extracts, 5 g of sample was weighed, diluted with ethanol and the research proceeded with ultrasonic shaking for 15 minutes. Then, the extract was filtered and raised in a 10 mL volumetric flask.

The ABTS assay was carried out according to the method established in the literature (Re et al., 1999). Briefly, the $ABTS^+$ stock solution was prepared from 7 mmol/L ABTS in potassium persulfate (2.45 mmol/L), and then incubated in dark for 16 h at room temperature. The $ABTS^+$ working solution was prepared by diluting the stock solution with ethanol to an absorbance of 0.70 ± 0.02 at 734 nm. All samples provide approximately 20-80% inhibition of blank absorbance. The 30 µL of the extract was mixed with 3.0 mL $ABTS^+$ working solution. The absorbance of the mixture was measured at 734 nm in spectrophotometer (Model UV-VIS, Kasuaki) after 25 min of incubation at 30 °C temperature, and the percentage of inhibition of absorbance at 734 nm was calculated. The standard curve was constructed by using Trolox (2000 –20 µmol/L) solution (Pertuzatti et al., 2014).

2.4 Instrumental color analysis

The instrumental determination of the color of the cereal bars was performed in a colorimeter (Model MiniScan EZ 4500L, Hunterlab) by using the CIELab reading scale, with CIE illuminant D_{65} (daylight) angle of 8° and standard observer CIE 10°. About 5 g of sample were placed in Petri dishes with 10.5 cm in diameter and 1.5 cm in height. The measured color parameters were: L*, a* and b*, where L* indicates luminosity and a* and b* represent the chromaticity coordinates. The staining measurements were expressed in terms of color angle value, H° (H° = $\tan^{-1}$ b/a), indicating the Hue angle (H°) of the sample (where 0° or 360° correspond to red, 90° corresponds to yellow, 180° corresponds to green and 270° corresponds to blue). Another parameter used was the chroma (C*) that represents the purity or intensity of the color. The color change (ΔE) of each sample was obtained by calculating the distance between two points in the three-dimensional space, defined by the parameters L*, a* and b*, according to Equation 1.

$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2} \quad (1)$$

where: ΔE= Color difference; ΔL= Difference in L* values; Δa= Difference in a*values; Δb= Difference in b* values.

2.5 Sensory analysis

Untrained consumers (108), of both sexes, ranging in age from 18 to 50 years were recruited based on the motivation and availability to participate in the research.

Sensory acceptability tests were performed in individual booths, where the samples were served in 15 cm disposable dishes, coded with random three digit numbers, along with a 200 mL glass containing water for mouth rinse between tasting sessions, and the analysis was carried out less than 24 hours after the processing of the cereal bars.

The research complied with the ethical principles of resolution 466 (Brasil, 2012) and was approved with the number of CAAE - 97070618.4.0000.5587.

In acceptance sensory analysis, a hedonic 9-point scale was used to evaluate the following parameters: color, flavor, aroma, texture and overall acceptability, whereas the purchase intention of the consumer was assessed using a 5-point scale.

The methodology to measure consumer expectation was divided into two sessions: in the first, the consumers evaluated the samples without any information about the product (blind test) and in the second, they evaluated the samples with information about the product (test with information). In this way, the comparison of the acceptance scores of the two sessions made it possible to measure the expectation generated by the cereal bar and to evaluate if the sensory characteristics corresponded to the expectations created.

2.6 Statistical analysis

Data analysis was carried out with ANOVA and Tukey's test focusing on significant differences in means. The analysis was performed by using Statistic 7.0 software program with significance level between mean differences at 10% ($p < 0.10$) for sensory analysis, due to greater variability of results since the tests employed used untrained judges, and 5% ($p < 0.05$) for other analyses. All analyses were made in triplicates and the results were given as means.

3 Results and discussion

Figure 2 shows the kinetic curves of the drying of the almonds, obtained through dehydration in gas-operated tray dryers, at three different temperatures besides the adjustment of the data to the logarithmic model.

In the curves obtained in the drying kinetics of the almonds, it was observed that the results found were the expected ones,

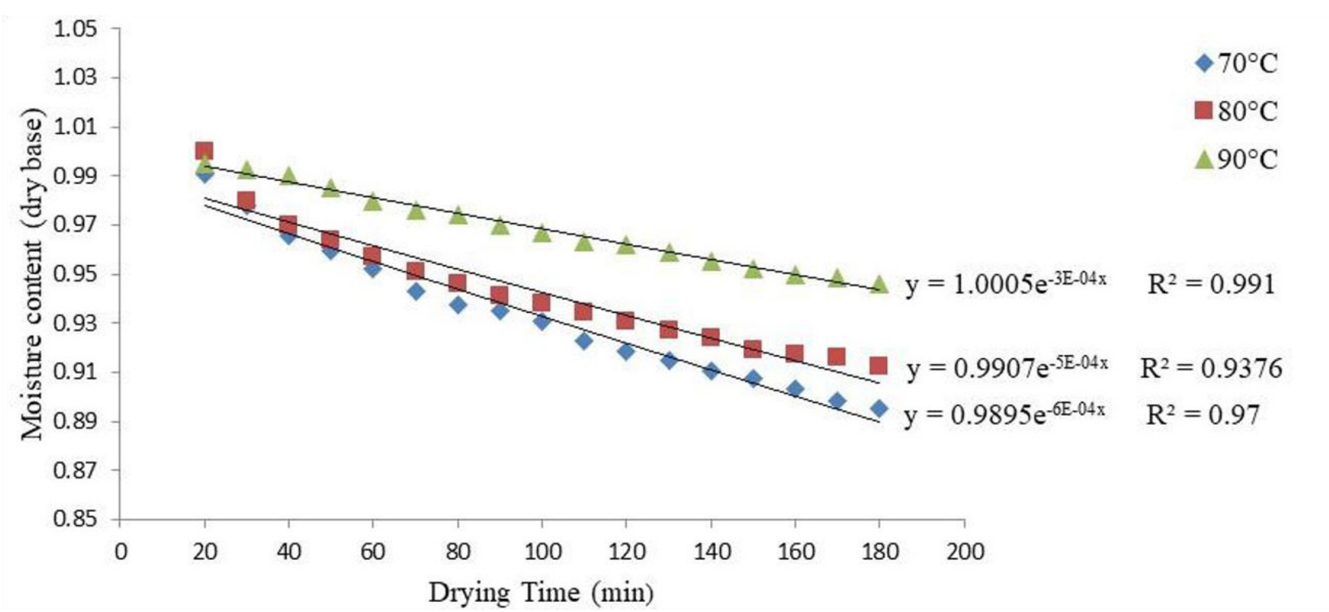

Figure 2. Drying curves of *pequi* almonds as a function of temperature.

where the drying time is inversely proportional to the temperature. Experimental data adjustment to the logarithmic model at the three drying temperatures reveals that the curve obtained at the temperature of 90 °C was the one that had a better projection to the model, showing $R^2 > 99\%$. Thus, the almonds were dehydrated at a temperature of 90 °C, since it was considered ideal to optimize the almond drying process.

The results obtained for the physicochemical analyses of *pequi* almonds and cereal bars are shown in Table 2.

Pequi almonds presented pH 6.7 and the cereal bars had pH between 5.03 and 5.52, with formulation A (12.5% of *pequi* almonds) differing statistically from the others at $p < 0.05$. Arévalo-Pinedo et al. (2013) obtained similar values of pH (5.38 and 5.39) when studying cereal bars of babassu flour. Regarding the soluble solids content, *pequi* almond presented 2.69° Brix and cereal bars 6.62° Brix.

In order to determine the antioxidant capacity of *pequi* almond, the ABTS method was chosen, which is an indirect method based on single electron transfer reactions, such as FRAP and DPPH, other widely used methods. However, due to the high fat content of *pequi* almond (Table 1), which could contribute to the extract not adequately dissolving in the aqueous phase used in the ferric reducing antioxidant power (FRAP) method and due to the fact that other fruits from cerrado (Savazzi et al., 2019) present high results when evaluated by the ABTS, when compared to DPPH, *pequi* almond was evaluated by ABTS.

However, *pequi* almonds presented low antioxidant capacity with the ABTS method (1.59 ± 0.08 μmol Trolox/g) when compared to studies evaluating the antioxidant capacity of *pequi* pulp (986.94 - 1154.42 μmol Trolox/g) (Leão et al., 2017). This fact may be related to the carotenoid content present in the pulp, which gives it an intense yellow color, while the almond has a white coloration. In relation to the antioxidant capacity of the cereals bar, there was an increase when compared to *pequi* almond, probably due to the presence of other ingredients known for their high antioxidant capacity such as honey (Batista et al., 2016) and grape (Barcia et al., 2015).

Pequi almond obtained a moisture content of 5.89%. In their work, Rabêlo et al. (2008) found a moisture content of 5.26% for

Table 2. Physicochemical analyses of *pequi* and cereal bars (Formulation A, B and Standard Formulation).

Parameters (%)	Formulation A	Formulation B	Standard Formulation	Pequi
pH	5.0 ± 0.1b	5.5 ± 0.1a	5.5 ± 0.1a	6.7 ± 0.1
Soluble Solids#	6.61 ± 0.04a	6.65 ± 0.02a	6.62 ± 0.12a	2.73 ± 0.11
ABTS*	2.45 ± 0.31a	2.22 ± 0.34a	2.11 ± 0.29a	1.59 ± 0.08
Moisture	6.701 ± 0.413b	6.702 ± 0.494b	9.165 ± 0.941a	5.891 ± 0.004
Ash	1.001 ± 0.005a	1.502 ± 0.002a	0.970 ± 0.892a	3.501 ± 0.502
Fiber	3.36 ± 0.05a	3.68 ± 1.50a	3.00 ± 1.00a	27.99 ± 1.40
Protein	11.91 ± 0.63a	13.12 ± 1.54a	10.90 ± 2.27a	29.62 ± 0.53
Lipids	15.14 ± 2.35a	17.31 ± 2.60a	13.21 ± 0.03a	35.36 ± 0.31
Carbohydrates**	10.63 ± 0.72a	10.81 ± 0.12a	9.86 ± 1.96b	2.00 ± 0.04
Fatty acids profile (%)				
C12	-	-	-	0.05
C14	-	-	-	0.31
C16	-	-	-	27.43
C16:1	-	-	-	0.35
C17	-	-	-	0.03
C18	-	-	-	2.23
C18:1	-	-	-	57.65
C18:2	-	-	-	3.73
C18:3	-	-	-	3.87
C20	-	-	-	0.26
C20:3	-	-	-	0.37
C20:5	-	-	-	3.45
C24	-	-	-	0.12
C24:1	-	-	-	0.15
% Saturated	-	-	-	30.43
% Unsaturated	-	-	-	69.57

#°Brix; *μmol Trolox/g sample; **no polysaccharides were quantified.

toasted *pequi* almond, similar to the value found in the present study. The low moisture content of *pequi* almond contributed to a decrease in the humidity of the almond cereal bars in the formulation, since formulation A (12.5% *pequi* almond) and formulation B (25% *pequi* almond) presented a moisture content of 6.7% differing significantly from the standard formulation, which obtained 9.16% moisture. All cereal bar formulations are in accordance with the standard defined by RDC No. 263, concerning Cereal Products, Starches, Flours and Bran (Brasil, 2005), with a maximum moisture content of 15%.

The *pequi* almond was found to be a source of high ash, fiber, protein and lipid content, 3.5%, 27.99%, 29.62% and 35.36%, respectively. However, there was no significant difference at $p < 0.05$ between the cereal bar formulations with and without the addition of *pequi* almond.

The fatty acid profile of *pequi* (Table 2) was composed of monounsaturated fatty acids (58.15%), saturated fatty acids represented 30.43% and polyunsaturated fatty acids (11.42%). The main compound in *pequi* is oleic acid (57.65%), followed by palmitic acid (27.43%), which is very close to the content identified by Cruz et al. (2020) in *buriti* oil. Other fatty acids that stand out are polyunsaturated fatty acids (PUFA) such as linolenic acid (3.87%), linoleic acid (3.73%), and eicosapentaenoic acid (EPA) (3.45%) which, like other omega-3 PUFAs, are found mainly in marine fish and fish oil and is an important component of the human cell membrane, regulating the inflammatory response.

Color is one of the most important quality parameters in food products, as it is the first characteristic that the consumer observes, resulting in acceptance or rejection of food. The data referring to the instrumental evaluation of the color of the cereal bars is in Table 3, where it can be observed that the cereal bars did not present significant difference at $p < 0.05$, for the color parameters L*, b*, as well as for parameter C* (Chroma), which represents the purity or intensity of the color and for the Hue angle values (H °), which indicates the color tone.

According to the color distinction (ΔE), it can be observed that the treatments presented differences that are perceptible to the human eye. They were classified as distinguishable when the standard formulation was compared with formulation B (value of ΔE 1.74) or when the standard formulation was compared to formulation A with a value of 2.58. When the formulations containing *pequi* were compared (Formulation A and formulation B), they were classified as easily distinguishable, as they presented a ΔE value of 3.4.

Table 4 shows the means obtained for the sensory attributes of cereal bars when the blind test was performed, that is, when consumers did not have information about the product they were evaluating. It was observed that there was no significant difference at $p < 0.10$ between the three formulations when the color and purchase intention by the consumer were evaluated. While for the attributes texture, flavor and overall impression, formulation B (25% of *pequi* almond) differed significantly, always presenting a mean lower than the other formulations. However, all formulations showed values ranging from "slightly liked" to " liked very much", which resulted in acceptance percentages ranging from 80.25% (texture of formulation B) to 86.83% (texture of formulation A).

In the second session of the sensory analysis, the test with information was carried out, evaluating the attributes global impression and purchase intention, for standard and formulation A (12.5% of *pequi* almond), as these obtained the best averages in the blind test.

Table 3. Luminosity values (L*), chromaticity coordinates a*, b*, C* and H° of the cereal bars.

Formulation	L*	a*	b*	C*	H°
Standard	55.34 ± 1.06a	7.71 ± 0.47ab	32.55 ± 1.83a	33.46 ± 1.65a	1.33 ± 0.01a
A	53.41 ± 2.80a	7.70 ± 0.62b	30.82 ± 0.52a	31.63 ± 1.35a	1.34 ± 0.01a
B	54.40 ± 1.42a	8.40 ± 0.32a	33.84 ± 1.05a	34.87 ± 1.15a	1.32 ± 0.02a

L (0) = black, 100 = white); a (+a = red (+60), -a = green (-60)); b (+b = yellow (yellow (+60), -b=blue (-60)). Averages followed by different lowercase letters in the same column indicate that there is a significant difference at $p < 0.05$ with Tukey's test.

Table 4. Scores for each cereal bar attribute in the blind test and the information test.

Formulation	Attributes				
	Color	Texture	taste	Overall impression	Purchase intention
Blind Test					
Standard	7.52 ± 1.23a	7.52 ± 1.38ab	7.84 ± 1.05a	7.77 ± 0.99a	4.29 ± 0.94a
Formulation A	7.49 ± 1.25a	7.80 ± 1.03a	7.80 ± 1.04ab	7.70 ± 1.06ab	4.32 ± 0.93a
Formulation B	7.50 ± 1.19a	7.23 ± 1.40b	7.49 ± 1.25b	7.44 ± 1.12b	4.26 ± 0.85a
Information test					
Standard	-	-	-	7.50 ± 1.24b	4.32 ± 0.95b
Formulation A	-	-	-	8.03 ± 0.95a	4.56 ± 0.84a

Means followed by the same letter on the same row did not differ significantly with Tukey's test ($p < 0.10$).

In the test with information, it was observed that being aware of the presence of *pequi* in the cereal bars contributes to a better acceptance and purchase intention of the consumers, since the formulation with *pequi* (formulation A) presented a higher acceptance average (8.03 for overall impression and 4.56 for purchase intention) compared to the mean in the blind test (7.70 for overall impression and 4.32 for purchase intention) and presented a higher mean than the standard formulation, differing significantly at $p < 0.10$. This fact is of great importance when demonstrating the valorization of a new product elaborated with a typical fruit of the Brazilian Cerrado, as it makes it easy to be inserted into the market, thus guaranteeing good commercialization.

Another interesting finding regarding the acceptance of cereal bars with and without *pequi* when compared with the test with and without information about the product was that for the standard cereal bar (without *pequi*) the information negatively affected the acceptance of the consumers. This fact was confirmed when the t test was carried out, obtaining a value of -2.01, with a significant p value (0.0464). While for the formulation with *pequi* (formulation A) the value of t was +3.12, demonstrating that the information had a positive influence with a significant p value (0.0023). Regarding the purchase intention, only the formulation with *pequi* (formulation A) had a significant p value (0.0023) and the value of t also showed that the influence of the information was positive for consumers (+3.13). While in the blind test, 48.15% of consumers stated that they would certainly buy the cereal bar with *pequi*, when they were informed of the presence of *pequi* in the bar this percentage rose to 62.04% of consumers.

In Table 5, the moisture content for each formulation over a period of 14 days is presented for determining shelf-life of the cereal bars. It is observed that only the standard formulation presented an increase of moisture, from the seventh day of storage on. This increase of moisture can cause modifications in the texture of the cereal bars, making them softer, and also contributes to the development of micro deteriorating organisms and for the development of oxidative rancidity, since the product has a high fat content, thus reducing the useful life of the product. For the other formulations there was no significant difference in the moisture content along the product storage.

4 Conclusion

Pequi presented a high ash, protein, fiber and lipid content, composed mainly of oleic acid (57.65%), followed by palmitic acid (27.43%), and a low antioxidant capacity. The best temperature for drying the almond was 90 °C. When comparing the cereal bars with and without *pequi*, they were considered distinguishable by the instrumental analysis of color. However, by the sensory analysis through the blind test, there was no significant difference between the cereal bars for color and consumer purchase intention. However, through the sensory test with information about the presence of *pequi* in the cereal bars, it was observed that being aware of it caused an increase in the acceptance and intention of purchase of the cereal bars, demonstrating that the consumption of new products with *pequi* is well valued by consumers.

Table 5. Moisture of cereal bar formulations during the 14-day shelf-life.

Parameter	Day	Formulation		
		Standard	A	B
Moisture (%)	1	6.24 ± 0.14b	6.62 ± 0.14a	6.51 ± 0.14a
	7	6.48 ± 0.00a	6.73 ± 0.00a	6.79 ± 0.00a
	14	6.55 ± 0.1ª	6.79 ± 0.10a	6.68 ± 0.10a

Means followed by the same letter on the same row did not differ significantly with Tukey's test ($p < 0.05$). A = Formulation A; B= Formulation B.

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