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Development of novel plant-based milk based on chickpea and coconut



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ABSTRACT

Lately, the demand for cow's milk substitutes has been growing. These substitutes are called plant-based milk, which are water-soluble extracts based on vegetables, legumes, cereals, pseudocereals, or nuts. Legumes are an attractive option in the development of new products since they are rich in proteins. Chickpea is one of them also rich in fibers, and minerals while coconut milk is plant-based, rich in lipids, and with good acceptance by consumers. Despite the use of coconut milk in food industries, there are no studies in the literature regarding the development of plant-based milk based on chickpea. So, this study aimed to develop a plant-based milk based on chickpea and coconut as a substitute for cow's milk. Seven different concentration extracts were prepared, with varying dilutions of chickpea and coconut extracts. The chemical composition was analyzed, including calcium, potassium and sodium content; total soluble solids; pH; titratable acidity; color, physical stability, and acceptance. Protein content ranged from 2.1 g/100 g to 1.04 g/100 g, and calcium content ranged from 107.41 to 131.26 mg/100 g among the seven samples. The samples containing 100, 90, 80, and 70% of chickpea extract reached higher protein values than other plant-based milk and calcium content similar to cow's milk, as well as soy milk, almond extract, and rice extract. Plant-based milk consumers well-accepted chickpea extracts with 10 and 30% of coconut. When the vanilla extract was added to both evaluated beverages in the secondary sensory analysis, acceptance improved. Novel plant-based milk based on chickpea and coconut was developed in this study. The drink has a good nutritional composition (such as protein, carbohydrate, lipid, and calcium content) when compared to cow's milk and other common substitutes for cow's milk, such as oat, almond, and rice extracts. Thus, it may be a potential substitute for cow's milk.

1. Introduction

In the past few years, consumers have become more interested in products that offer benefits to health (Jaekel, Rodrigues, & Silva, 2010). Similarly, there is a group of products with great interest in the market, which is cow's milk substitutes. The need for alternatives for cow's milk has stood out mainly because of pathological reasons and food practices such as vegetarianism and veganism (Jeske, Zannini, & Arendt, 2017b). The strategy used by these individuals and the food industry has been the plant-based milk. It is essential to mention that there are alternatives for individuals with lactose intolerance, such as lactose-free products and the use of lactase enzyme, however, for allergic individuals and vegans, the only choice is plant-based milk (Vanga & Raghavan, 2018).

The production and commercialization of plant-based milk have grown exponentially worldwide (Sethi, Tyagi, & Anurag, 2016). In Europe, the market of these beverages grew 9% in 2015 with a variety

of 138 types (Jeske, Zannini, & Arendt, 2017a). In the United States, an intensive decrease in cow's milk consumption has been observed along with the increased demand for non-dairy products (Singhal, Baker, & Baker, 2017). There is no data in the literature about the increase in consumption of plant-based milk in Brazil, but media research points to a 51.5% increase in the consumption of these beverages in 2018 (MilkPoint, 2019).

Plant-based kinds of milk are water-soluble extracts based on oil-seeds, cereals, pseudocereals, seeds and/or legumes. Moreover, the production steps of these extracts are mostly the same. The raw material is previously soaked for a few hours, and then processed with water. Then, the extract is filtered to remove the insoluble residues. Other ingredients, such as flavors, sugar, and stabilizers may be added. Stability, homogenization and pasteurization processes are performed at the end of the production flow, resulting in liquid extracts that can be colloidal suspensions or emulsions (Jeske et al., 2017a; Makinen, Uniacke-Lowe, O'mahony, & Arendt, 2015).

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The nutritional properties of plant-based milk will depend on the quality and type of the used raw material, and the type of processing performed (Makinen, Wanhalinna, Zannini, & Arendt, 2016). Some of these extracts, like those produced with almond, rice, and oats contain minimal amounts of protein, calcium, and iron when compared to cow's milk (Makinen et al., 2016; Singhal et al., 2017). Therefore, a common disadvantage of most plant-based milk is the low protein content (Jeske et al., 2017b). In the study by Jeske et al. (2017a), half of the analyzed samples displayed less than 0.5% protein, and only soy extract (2.6%) presented similar values compared to cow's milk (3.7%). It is important to emphasize, however, that approximately 14% of patients who are allergic to cow's milk also have reactions due to sov consumption (Jeske et al., 2017b). Plant-based milk are lactose and cow's milk protein-free. but many of these substitutes are based on allergenic ingredients, such as soy, almonds, peanuts and kidney bean (Jeske et al., 2017b; Paul, Kumar & Sharma, 2019; Vojdani, Turnpaugh, & Vojdani, 2018). Thus, other options must be considered to exclude allergens and include more consumers.

Most industrialized plant-based milk is fortified with calcium and vitamin D, but the bioavailability of these nutrients in fortified products has not been sufficiently studied (Singhal et al., 2017). Also, to improve sensory and technological characteristics, many manufacturers use low-cost additives, such as sweeteners, sugars, and stabilizers, implying a beverage with impaired nutritional value. Thus, the food industry needs to improve the manufacturing process, from choosing suitable quality materials and ingredients (Jeske et al., 2017b).

Hence, the development of new substitutes for cow's milk with a similar composition is necessary. Moreover, these substitutes must be accessible to consumers (low cost and easy to find), pleasant, and allergenic free.

In this sense, legumes are a versatile and attractive option in the development of new products, since they are known to be rich in proteins (Chandra-Hioe, Wong, & Arcot, 2016). Chickpea (Cicer arietinum L.) is a legume widely consumed around the world, which is rich in proteins, fibers, and minerals like zinc, calcium, and magnesium. The protein content in chickpea varies from 21% to 25% (Rachwa-Rosiak, Nebesny, & Budryn, 2013). There are still no officially registered allergens in chickpea, as there are for soybeans and peanuts (Cabanillas, Jappe, & Novak, 2017).

The developed chickpea extract has a darker and yellowish color when compared to cow's milk. Besides, chickpea extract has a "beany" flavor (Jeske et al., 2017b). This "beany" flavor is a characteristic of the legume group. It is due to its composition of antinutritional compounds, such as saponins and isoflavones (Carrão-Panizzi, Beléia, Prudêncio-Ferreira, Oliveira, & Kitamura, 1999). Thus, blends with other compounds may be one way of developing more pleasant beverages. An option of plant-based milk with milk-like color and desirable sensorial characteristics is coconut milk. Since coconut has a white color and it presents more lipids when compared to chickpea, it is a good source to be blended to the chickpea extract.

Thus, the blend of chickpea and coconut may be an exciting and viable option to obtain good nutritional plant-based milk with desirable aroma, flavor, and color characteristics since sensorial quality is one of the most critical determinants in the decision to purchase a product (Jaekel et al., 2010).

There are no studies in the literature regarding the development of pure chickpea extract nor chickpea extract associated with coconut. Due to the high protein content of this legume, it is interesting to evaluate its use in the production of new alternatives for consumers to replace cow's milk. Therefore, different concentrations of chickpea and coconut extracts were combined to find the best blend, according to nutritional composition and acceptability parameters.

2. Materials and methods

2.1. Preparation of the samples

The plant-based milk preparation was carried out in the Dietetic Technique Laboratory, University of Brasilia, Brazil, and the characterization step was performed at the Food Analysis Laboratory, University of Brasilia, Brazil. The chickpea used was the cultivar BRS Toro FLIP 06–155C, produced in 2018 in the experimental area of EMBRAPA HORTALIÇAS, Distrito Federal, Brazil. The chickpea was provided by EMBRAPA, and 5.0 kg of the dried grain was used for all tests and stages. For the preparation of the chickpea extract (CPE), the raw grain was soaked for 12 h, and the water was discarded to decrease antinutritional factors. Then, the grains (1.0 Kg of chickpea for each stage of preparation of the samples) were cooked with 2.5 L of water (room temperature) in a domestic pressure cooker of 7 L of capacity (Rochedo®) at 120 °C and 2.0 atm for 20 min after pressure started.

After this step, the chickpea was separated from the cooking water, which was reserved for further use. The chickpea peel has not been removed. The grains were processed in Thermomix® at rate 8 for 1 min with the remaining water added by filtered water in the ratio of 1:4 (1 part of raw chickpea grains: 4 parts of water). For that, it was used the remaining water (500 mL) of the cooking process with additional filtered water (19.5 L) to complete this volume. The resulting material (liquid) was filtered with a cloth (tissue strainer). The material trapped by the tissue strainer was discarded. The production flowchart of the chickpea extract is in Fig. 1.

Mature coconut was used in all the tests and steps, and it was purchased in the local market in October of 2018 in Brasilia, Brazil. It was used 5.0 kg of mature coconut (using the shell – inner hard coat of fruit and the meat – solid endosperm) for the development of all the extracts. For the preparation of the coconut extract (CNE), it was used the proportion of 1:3 (1 part of mature coconut: 3 parts of boiling water). Boiling filtered water was used to facilitate the extraction of coconut components into the extract. The coconut and the water were processed in an ordinary blender (Philco*, model PH900) at medium speed for 2 min, and the resulting material was filtered with a cloth (tissue strainer) to obtain the coconut extract, which is the liquid form.

The elaborated samples were as follows: (i) 100% CPE; (ii) 90% CPE and 10% CNE; (iii) 80% CPE and 20% CNE; (iv) 70% CPE and 30% CNE; (v) 60% CPE and 40% CNE; (vi) 50% CPE and 50% CNE; (vii) 100% CNE. After preparing all the samples in the laboratory, they were put in the fridge at 4 °C for further sensory analysis on the same day.

2.2. Chemical composition

All analyses were performed in triplicate. For the determination of moisture content, the Analytical Standards of the Instituto Adolfo Lutz (2008) was used in two stages. The first step consisted of heating the samples in an oven at 50 °C until constant mass. Then, 2 g of each sample was heated in an oven at 105 °C until constant mass. The determination of the protein content of samples was performed according to AOAC (2005), method 991.22. The total lipid content was determined by the extraction method Am 5-04 using Ankom Technology's XT15 Extractor (AOCS, 2005). The determination of the ash content of the samples was performed by the method 945.45 (AOAC, 2005). Total carbohydrates content present in the samples was determined by difference, subtracting from 100 the values found for moisture content, protein content, lipid content, and ash content, according to method 986.25 (AOAC, 2005).

The concentration of sodium (Na), potassium (K), and calcium (Ca) were obtained in a flame photometer AP-1302 (Labnova®, Santo André,

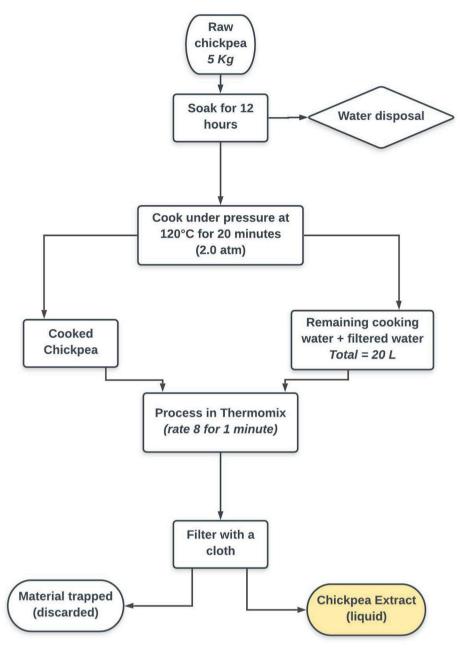


Fig. 1. Production flowchart of chickpea extract.

Brazil), according to method 956.01 (AOAC, 2005). The equipment was calibrated using standard solutions of the analyzed minerals (Na, K, and Ca), according to the manufacturer's recommendations (Labnova®). Results were expressed as mg 100 g $^{-1}$.

2.3. Determination of color

The color evaluation was performed with the aid of the ColorQuest XE Spectrophotometer (HunterLab*, Reston, United States) obtaining the values of the coordinates L* (measurable in terms of white to black intensity), a* (measurable in terms of red and green intensity) and b* (measurable in terms of yellow and blue intensity) of the Hunter system. With the values of the coordinates, a* and b*, the hue angle (h*, Equation (1)), and color saturation or chroma (C*, Equation (2)) were obtained (Francis, 1975; Mclellan, Lind, & Kime, 1995).

$$h * = arctan(b * /a *)$$

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

Equation 2

2.4. Total soluble solids content, pH and titratable acidity

Total soluble solids content (TSS) was measured in a table refractometer (ATAGO*, model 1 T), using two drops of each homogenized sample. The results were expressed in 'Brix. The pH was determined by direct measuring in a digital pH-meter (Digimed*, model DM21), with 10 g of each sample, according to the Analytical Standards of the Instituto Adolfo Lutz (2008). For the titratable acidity analysis, 10 g of each sample was previously diluted in 100 mL of distilled water (Instituto Adolfo Lutz, 2008). For the titration, a standard solution of 0.1 N NaOH and phenolphthalein indicator were used.

2.5. Physical stability

The physical stability of the seven samples was evaluated for 72 h at

Equation 1

Chemical composition and concentration of sodium (Na), calcium (Ca) and potassium (K) of the plant-based extracts made from chickpea and coconut produced in different proportions and cow's milk, soy milk, almond

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Extracts	Moisture (g/100 g)	Ash (g/100 g)	Protein (g/100 g)	Lipid (g/100 g)	Carbohydrates (g/100 g)	Na (mg/100 g)	Ca (mg/100 g)	K (mg/100 g)
100% CPE	$93.75^a \pm 1.5$	$0.37^{a} \pm 0.11$	$2.1^{a} \pm 0.07$	$0.39^{c} \pm 0.22$	$3.39^{a} \pm 1.29$	$1.19^{c} \pm 0.29$	$131.26^{a} \pm 14.13$	$206.99^{abc} \pm 38.4$
90% CPE + 10% CNE	$92.94^{a} \pm 1.86$	$0.4^{a} \pm 0.11$	$2.09^{a} \pm 0.16$	$1.08^{\circ} \pm 0.28$	$3.49^{a} \pm 1.52$	$1.60^{\circ} \pm 0.36$	$138.78^{a} \pm 18.64$	$231.6^{a} \pm 36.97$
80% CPE + 20% CNE	$93.63^{a} \pm 0.7$	$0.33^{a} \pm 0.05$	$1.96^{ab} \pm 0.07$	$1.74^{\circ} \pm 0.35$	$2.33^{a} \pm 0.78$	$1.79^{\circ} \pm 0.92$	$121.35^a \pm 21.34$	$185.55^{\text{abc}} \pm 3.97$
70% CPE + 30% CNE	$91.77^{ab} \pm 1.79$	$0.32^{a} \pm 0.08$	$1.9^{ab} \pm 0.18$	$3.25^{b} \pm 1.15$	$2.77^{a} \pm 1.46$	$2.53^{\circ} \pm 1.27$	$107.41^a \pm 9.68$	$175.98^{abc} \pm 18.67$
60% CPE + 40% CNE	$91.34^{ab} \pm 1.24$	$0.43^{a} \pm 0.09$	$1.73^{\rm bc} \pm 0.05$	$3.29^{b} \pm 0.6$	$3.21^{a} \pm 1.2$	$2.04^{\circ} \pm 0.53$	$132.02^a \pm 21.76$	$217.26^{abc} \pm 8.01$
50% CPE + 50% CNE	$92.19^{ab} \pm 0.41$	$0.33^{a} \pm 0.07$	$1.54^{\circ} \pm 0.09$	$3.43^{b} \pm 0.38$	$2.52^{a} \pm 0.12$	$5.58^{b} \pm 1.97$	$110.53^a \pm 8.96$	$167.8^{bc} \pm 58.77$
100% CNE	$90.55^{b} \pm 1.75$	$0.32^{a} \pm 0.09$	$1.04^{\rm d} \pm 0.31$	$7.42^{a} \pm 1.68$	$0.67^{b} \pm 0.2$	$8.55^a \pm 1.79$	$110.54^{a} \pm 10.48$	$156.56^{\circ} \pm 26.27$
Cow's milk*	88.13****	ı	3.39	2.12	4.66	1	127	1
Soy milk*	1	1	2.92	1.67	1.67	ı	125**	I
Almond extract*	97.05****	1	0.42	1.04	0.42	ı	188**	
Rice extract*	89.28****	ı	0.0	1.04	4.58	ı	104***	ı

CPE-chickpea extract; CNE - coconut extract; *National Nutrient Database for Standard Reference (USDA, 2019); ** fortified with Calcium carbonate; *** fortified with Tri-calcium phosphate. < 0.05Means followed by the same letter in columns do not differ by Fisher's test (p **** Chalupa-Krebzdak et al. (2018).

All analysis in triplicate

4 °C through the phase separation analysis (Zaaboul, Raza, Cao, & Yuanfa, 2019). Samples containing 10 mL of the extracts were stored in 15 mL falcon tubes and assessed at every 24. The falcon tubes had volume marking. Thus it was possible to obtain the volumes of the phases during the 72 h. The analysis included observing the phase separation volume every 24 h.

2.6. Sensorial analysis

The research project was previously submitted and approved by the Ethics Committee of the University of Brasilia (88754618.4.0000.0030/2018).

The sensory analysis was performed at the Dietetic Technique Laboratory and the Food Analysis Laboratory of the University of Brasilia, in two steps: the first one with the overall participants and the second one with a specific group of plant-based milk habitual consumers.

In the first step, samples 1 to 6 were evaluated by 128 untrained assessors, randomly presented. The mean age of the assessors was 24 years old, and 60% were female. The samples were: (i) 100% CPE; (ii) 90% CPE and 10% CNE; (iii) 80% CPE and 20% CNE; (iv) 70% CPE and 30% CNE; (v) 60% CPE and 40% CNE; (vi) 50% CPE and 50% CNE. The sensory analysis was performed only with the samples that contained chickpea extract, that is, the sample 100% CNE was not included in this evaluation since the study had as base the chickpea and coconut was added to improve sensory characteristics.

Assessors received approximately 25 mL of each sample in a refrigerated temperature and randomized order in white plastic cups with three-digit code numbers. They rated them using a structured 9-point hedonic scale to evaluate overall impression, color, odor, taste, and texture (9 – "like extremely" to 1 – "dislike extremely"). Samples were prepared and served on the same day refrigerated to guarantee assessors' safety. All pieces of equipment and materials used to prepare samples were sterilized before their use.

Before the test, assessors were invited to participate and sign a Consent Form. Participants also filled out a questionnaire comprising sociodemographic questions and information related to plant-based milk consumption habits. The inclusion criteria for assessors' participation were: being 18 years old or more, and the absence of allergic or intolerance reactions to coconut and/or chickpea. In addition to the samples, participants received a glass of water, an empty cup for sample disposal, napkins, and salt biscuit to eat between samples analysis.

After the first step of the sensorial analysis and based on its results, the second step of the sensorial analysis was conducted with stricter inclusion criteria for assessors. The researchers looked for consumers of plant-based milk at least biweekly, and participants who like these products (provide score six or higher in the hedonic scale of the recruitment form). In this analysis, 28 participants with mean age 29 years old and 71% female evaluated four coded samples, which were: 90% CPE and 10% CNE; 70% CPE and 30% CNE; 90% CPE and 10% CNE plus 0.3% vanilla extract; 70% CPE and 30% CNE plus 0.3% vanilla extract. The vanilla extract was added to assess whether flavoring the samples improves beverage acceptance. Samples with 90% CPE and 10% CNE; 70% CPE and 30% CNE were chosen because their protein content did not differ statistically, which was one of the researchers' aims.

Another way to evaluate the acceptance of a product is by analyzing the acceptance percentage, in which the panelists are divided into three groups: acceptance (scores equal or greater than 6), indifference (scores equal to 5), and rejection scales (scores equal or less than 4). A product is considered accepted when the acceptance percentage is greater than or equal to 70% (Minim, 2013, p. 332).

2.7. Statistical analysis

A completely randomized design with three replicates was adopted.

In the physical stability evaluation, a 7x3 factorial scheme was adopted, with seven extracts and three storage periods (24, 48, and 72 h). Data on chemical composition, micronutrients, color, and the first sensorial analysis were submitted to ANOVA, and significant results were analyzed by Fisher's Least Significant Difference (LSD) test at 5% probability in the XLSTAT* program. Data of second sensorial analysis were submitted to student t-test between two means at 5% probability.

3. Results and discussion

3.1. Chemical composition

Chickpea was the chosen raw material due to its protein content, ranging from 20.9 to 25.27% (Rachwa-Rosiak et al., 2013). It was decided to include the coconut milk to the chickpea extract, due to its good acceptability by the population and its lipid content and color (Marina & NurulAzizah, 2014; Scholz-Ahrens, Ahrens, & Barth, 2019). According to Araujo, Botelho, Pilla, and Borgo (2014), cow's milk presents its white color because of the light reflection of the lipids, calcium phosphate, and casein. Also, fat is responsible for the softness, aroma, and taste of milk. Therefore, the development of samples with coconut would increase the lipid content and change organoleptic characteristics.

The chemical composition and concentration of sodium (Na), calcium (Ca) and potassium (K) of the samples elaborated with chickpea and coconut are presented in Table 1. These were compared with literature data of cow's milk, soy milk, almond extract, and rice extract.

Protein values ranged from 2.1 g/100 g (100% chickpea extract, CPE) to 1.04 g/100 g (100% coconut extract, CNE) among samples. A higher proportion of CPE in the beverage conducted to higher protein content, while a higher proportion of CNE decreased the protein content. Regarding the extract with 100% CPE, a statistically significant difference (p < 0.05) was observed only in comparison to samples with 40, 50, and 100% CNE. The lowest value for protein content was found for the sample containing 100% CNE, which was already expected since coconut is not a protein food source (Patil & Benjakul, 2018).

In studies conducted by Jeske et al. (2017a) and Singhal et al. (2017) with commercial coconut milk, protein values were lower than 1.0 g/100 g. Another study carried out by DebMandal and Mandal (2011) pointed out a total of 1.6 g/100 g protein in fresh coconut milk. In this research, fresh coconut milk was used, and the protein value was 1.04 g/100 g.

The nutritional composition of coconut milk will depend on the quality of the coconut meat and the efficiency of milk extraction. Patil and Benjakul (2018) said in their review study that different proportions of water and coconut meat did not seem to affect the extraction of proteins and lipids from the coconut into coconut milk.

Lipid content values varied between samples; however, with no statistical difference among the extracts elaborated with 100% CPE, 90% CPE and 10% CNE, and 80% CPE and 20% CNE (p > 0.05). All samples were significantly different from the extract of 100% CNE. The lipid content of this sample (7.42 g/100 g) was 19-fold higher than extract 100% CPE. These results agree with data from the raw materials used in each sample. While in mature coconut, there are approximately 60% lipids (DebMandal & Mandal, 2011), in chickpea, lipid content is around 5% (Rachwa-Rosiak et al., 2013). Due to low nutrient density and very high content of lipids, coconut milk cannot be considered a good substitute for cow's milk (Scholz-Ahrens et al., 2019).

The purpose of the present study was to produce a good substitute for cow's milk. The sample containing 100% CPE, although it presents a high protein value, it did not show a good sensorial evaluation for color and taste.

Although cow's milk displays higher protein content (3.39 g/100 g), followed by soymilk, with 2.92 g/100 g, it should be noted that both of these products are allergenic (Kattan, Cocco, & Järvinen, 2011). The

beverage made from chickpea and coconut reached higher protein values than other plant-based kinds of milk, as it can be seen in Table 1. In a review study by Chalupa-Krebzdak, Long, and Bohrer (2018), they found average protein values of 0.76 g/100 g in almond extract, 1.28 g/100 g in coconut extract, 0.28 g/100 g in rice extract, 0.83 g/100 g in hemp extract, and 1.31 g/100 g in cashew extract. It is relevant to emphasize that they evaluated studies with commercial plant-based milk, and many of them fortified with proteins and other nutrients, like vitamin D and calcium (Makinen et al., 2015).

Many studies have been carried out to test new materials and to evaluate the nutritional composition of plant-based milk. An example was the study by Pineli et al. (2015), in which quinoa was tested. However, the developed beverage achieved a low protein content (1.7 g/100 g) when compared to cow's milk. Besides, this pseudocereal has a very high cost compared to other grains, such as beans, soy, and chickpeas.

Almond extract is one of the most widely consumed cow's milk substitutes (McCarthy, Parker, Ameerally, Drake, & Drake, 2017), mainly due to its flavor and taste (Vanga & Raghavan, 2018). Still, its protein content is 8-fold lower than that of cow's milk (3.39 g/100 g). In comparison to almond extract, the developed plant-based milk displays a 4.5-fold higher protein content. On the other hand, rice extract, although considered a cow's milk substitute, contains insignificant amounts of protein, with 0.3 g/100 g (Scholz-Ahrens et al., 2019). Furthermore, it is relevant to emphasize that the developed beverage attained these protein values without the need for additives or compounds that improve protein extraction.

In respect to calcium, a micronutrient related to bone health (Scholz-Ahrens et al., 2019), values may vary between plant-based milk. Chalupa-Krebzdak et al. (2018) found in their review study, average calcium values from 12 mg/100 g in hemp extract to 160 mg/ 100 g in almond extract. The developed plant-based milk, with levels ranging from 107.41 to 131.26 mg/100 g of calcium, represents a good substitute for cow's milk, as well as soymilk, almond, and rice extracts. Nevertheless, it is important to mention that soy, almond and rice extracts are usually fortified with calcium carbonate or tri-calcium phosphate by the food industry to make them comparable to cow's milk (Table 1). In cases in which non-fortified plant extracts replace cow's milk and if the diet is not balanced, there is a high risk of nutritional deficiencies, such as for protein and calcium (Scholz-Ahrens et al., 2019). Therefore, it is essential to have nutritional monitoring in cases of cow's milk substitution (Makinen et al., 2016). However, the beverage developed in this study obtained very similar calcium values to cow's milk without the need for fortification.

Many of the commercial plant-based milk are fortified with proteins and other nutrients, like vitamin D, calcium, and vitamin B12. These products are fortified to fight a nutrient deficiency in individuals who do not consume cow's milk and dairy products (Makinen, Wanhalinna, Zannini & Arendt, 2015), such as calcium deficiency. It is known that cow's milk is one of the primary sources of this micronutrient in feeding (Vanga & Raghavan, 2018).

Regarding sodium, the content found in the developed beverages were lower than the levels in cow's milk and commercial plant-based milk, mainly because the samples were developed by hand (small scale). It is known that the addition of food additives by the food industry greatly increases the levels of this micronutrient in food (Ning, Mainvil, Thomson, & Mclean, 2017). The food industry often adds sodium or its compounds to food to enhance the product's flavor (Kameník, Saláková, Vyskočilová, Pechová, & Haruštiaková, 2017).

It is also known that the use of acidified saline solutions can also increase protein extraction in plant-based milk. An example of this was the study of Pineli et al. (2015) in which quinoa was used to elaborate a plant-based milk. Acidified saline solutions were tested in different concentrations of sodium chloride (NaCl) and pH in place of water to increase the protein content in the developed extract. They found that the acidified saline solution in the cooking process managed to extract

Table 2

Data of color measures, total solids, pH and titratable acidity of the seven plant-based extracts made from chickpea and coconut produced in different proportions.

Extracts	Color			Total soluble solids (°Brix)	pH	Titratable acidity mL NaOH 1 mol $L^{-1}100~g^{-1}$	
	L*	h*	C*				
100% CPE 90% CPE + 10% CNE 80% CPE + 20% CNE 70% CPE + 30% CNE 60% CPE + 40% CNE 50% CPE + 50% CNE 100% CNE	$53.4^{\circ} \pm 0.97$ $62.13^{d} \pm 2.84$ $66.79^{cd} \pm 3.64$ $69.71^{bcd} \pm 3.74$ $72.11^{bc} \pm 3.47$ $73.94^{ab} \pm 3.56$ $78.91^{a} \pm 4.98$	$86.73^{a} \pm 0.34$ $85.59^{ab} \pm 0.79$ $85.5^{ab} \pm 0.61$ $85.78^{ab} \pm 0.9$ $86.18^{ab} \pm 0.92$ $86.54^{ab} \pm 0.79$ $84.94^{b} \pm 1.58$	$26.07^{a} \pm 1.47$ $26.35^{a} \pm 0.82$ $24.39^{ab} \pm 1.52$ $22.67^{bc} \pm 1.44$ $20.48^{cd} \pm 1.8$ $18.31^{d} \pm 1.68$ $5.47^{c} \pm 1.14$	$\begin{array}{l} 4.04^{a}\pm0.23\\ 3.94^{ab}\pm0.13\\ 3.8^{ab}\pm0.2\\ 3.62^{b}\pm0.21\\ 3.1^{c}\pm0.24\\ 2.88^{c}\pm0.11\\ 1.77^{d}\pm0.18 \end{array}$	$6.25^{a} \pm 0.44$ $6.39^{a} \pm 0.44$ $6.45^{a} \pm 0.48$ $6.5^{a} \pm 0.44$ $6.46^{a} \pm 0.38$ $6.45^{a} \pm 0.39$ $6.44^{a} \pm 0.28$	$\begin{array}{c} 1.39^{a} \pm 0.21 \\ 1.27^{a} \pm 0.19 \\ 1.27^{a} \pm 0.13 \\ 1.23^{a} \pm 0.17 \\ 1.25^{a} \pm 0.11 \\ 1.19^{a} \pm 0.08 \\ 0.78^{b} \pm 0.22 \end{array}$	

CPE-chickpea extract; CNE - coconut extract.

Means followed by the same letter in columns do not differ by Fisher's test (p < 0.05). All analysis in triplicate.

3-fold more protein for the beverage than the basic process with water (Pineli et al., 2015). In this plant-based milk based on chickpea and coconut, it was not necessary to use acidified saline solutions to increase the extraction of proteins, which also explains the low sodium content.

Sodium can interfere in blood pressure and renal overload, and thus, there must be control in its consumption (Kameník et al., 2017). Therefore, the sodium values found in the developed beverages are a positive point for its nutritional composition.

Concerning potassium, the values found in the samples are not similar to the potassium content of cow's milk (134 mg/100 g). The high levels found (231.6 mg in 100% CPE extract) can be explained due chickpea being a source of potassium, containing about 1116 mg/100 g in its composition in raw form (TACO, 2011). It is known that potassium is an essential nutrient and one of the most abundant micronutrients in intracellular fluid, playing a fundamental role in maintaining cellular function (Stone, Martyn, & Weaver, 2016).

3.2. Color

The mean values of L* (luminosity), hue angle (h*), and chroma (C*) for the different samples are shown in Table 2. It is essential to emphasize the importance of quantitative color evaluation concerning food quality. According to AMSA (2012, pp. 1–136), visual appraisals of color in foods are closely related to consumer or taster evaluations and set the benchmark for instrumental measurement comparison, such as those performed in the present study.

When the color of the samples was analyzed, significant variation was obtained (p < 0.05), when the variables L*, h*, and C* were analyzed. The most intense changes were observed when the variable L * was analyzed. The value of L* obtained for sample 100% CNE (78.91) was the highest and could be comparable to a UHT cow's milk (81.89), as analyzed in the study by Jeske et al. (2017a). Sample with 100% CPE was the darkest one (53.40), with a statistical difference from the others and very different from that found in other studies for cow's milk. The L measure increases as the concentration of CNE increases. Concerning the hue angle (h), there was only a significant difference between 100% CPE (86.74) and 100% CNE (84.94), with all values remaining in the first quadrant (0 \leq h \leq 90). As for chroma (C), as the percentage of CNE increased, there was a reduction in mean values. It is important to note that chroma is related to color purity, with higher levels indicating more intense or vivid colors (Maskan, 2001).

3.3. Total soluble solids content, pH and titratable acidity

Data on total soluble solids content, pH, and titratable acidity are described in Table 2.

Results of pH were similar between the samples, and there was no statistical difference. The pH values of all samples remained above 6.30. Regarding the titratable acidity, only 100% CNE was significantly

different from the others. In a study by Makinen et al. (2015), authors found a pH value of 6.83 for a cow's milk sample, which does not differ from the values found in the developed beverage.

The importance of determining variables such as pH and titratable acidity is emphasized, as they are important factors associated with the growth of microorganisms in food. In particular, pH influences the occurrence and distribution of microorganisms, and less acidic foods (p > 4.5) are more susceptible to the development of pathogenic microorganisms, in addition to molds and yeasts (Jin & Kirk, 2018; Roberts & Greenwood, 2003). Besides, it is known that protein extraction is improved in acid environments, that is, at lower pH (Pineli et al., 2015). This information is consistent with the values found for protein in the developed beverages.

3.4. Physical stability

There was no significant variation in the interaction between sample types and storage period when the physical stability was analyzed. However, there was a significant variation because of the nature of the sample (Fig. 2), with the observation of two phases. When the upper phase (mL/100 mL) was analyzed, the mean values increased as the percentage of CNE was reduced. It is noteworthy that in the samples

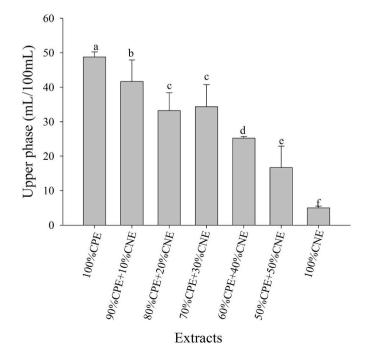


Fig. 2. The upper phase (mL/100 mL) of the plant-based extracts made from chickpea and coconut produced in different proportions.

Table 3First sensory evaluation of six different samples of chickpea and coconut plant extracts based on a 9-point hedonic scale.

Extracts	Overall	Color	Odor	Taste	Texture
100% CPE	4.4° ± 1.8	4.4° ± 1.9	$4.8^{a} \pm 1.6$	$3.8^{d} \pm 2$	5.3 ^b ± 2.1
90% CPE +10% CNE	$5.1^{\text{b}} \pm 1.7$	$5^{\rm b} \pm 1.7$	$5.1^{ab} \pm 1.5$	$4.2^{cd} \pm 2$	$5.8^{ab} \pm 1.7$
80% CPE +20% CNE	$5.1^{\text{b}} \pm 1.7$	$5.2^{b} \pm 1.7$	$5.1^{ab} \pm 1.4$	$4.4^{bc} \pm 2.1$	$5.9^{a} \pm 1.8$
70% CPE +30% CNE	$5.2^{ab} \pm 1.9$	$5.3^{ab} \pm 1.7$	$5.2^{a} \pm 1.4$	$4.5^{bc} \pm 2.1$	$5.9^{a} \pm 1.8$
60% CPE +40% CNE	$5.3^{ab} \pm 1.8$	$5.3^{ab} \pm 1.8$	$5.2^{a} \pm 1.5$	$4.8^{ab} \pm 2$	$5.9^{a} \pm 2.0$
50% CPE +50% CNE	$5.6^{a} \pm 1.8$	$5.6^{a} \pm 1.9$	$5.3^{a} \pm 1.7$	$5.1^{a} \pm 2.1$	$6.0^{a} \pm 2.0$

CPE-chickpea extract; CNE - coconut extract.

Means followed by the same letter in columns do not differ by Fisher's test (p < 0.05).

Table 4
Results of the second sensory analysis performed with two of the extracts developed, with and without the addition of vanilla extract.

Extracts	Overall	Color	Odor	Taste	Texture
90% CPE +10% CNE	$5.5^{a} \pm 2.3$	$5.6^{a} \pm 2.0$	$6.1^a \pm 1.8$	$4.9^{a} \pm 2.2$	$5.8^{a} \pm 2.1$ $6.4^{b} \pm 2.0$ $6.7^{A} \pm 1.8$ $6.5^{A} \pm 1.9$
90% CPE +10% CNE + VE	$5.7^{a} \pm 2.3$	$5.6^{a} \pm 1.9$	$6.8^b \pm 2.3$	$5.6^{b} \pm 2.4$	
70% CPE +30% CNE	$5.9^{A} \pm 2.1$	$6.5^{A} \pm 2.0$	$5.9^B \pm 1.8$	$5.6^{A} \pm 2.1$	
70% CPE +30% CNE + VE	$6.4^{A} \pm 1.7$	$6.4^{A} \pm 1.8$	$6.9^A \pm 1.8$	$6.0^{A} \pm 2.1$	

CPE-chickpea extract; CNE - coconut extract; VE - vanilla extract.

Means followed by the same lowercase letter for 90% CPE and 10% CNE extract and capital letter for 70% CPE + 30% CNE extract in columns do not differ by t-test (p < 0.05).

with at least 40% of CNE, the formation of precipitate in the lower phase was observed. Separation is expected since samples are emulsions that have not been submitted to any heat treatment, such as pasteurization and/or homogenization (Patil & Benjakul, 2018).

It is essential to mention that due to limitations, this was the methodology available while the research was being carried out.

3.5. Sensorial analysis

The consumers' evaluation of the six samples of the first step of sensory analysis is described in Table 3.

Concerning the results from the first sensory analysis, the best hedonic ratings were for 50% CPE and 50% CNE, while the worst was for 100% CPE. Despite the best scores for 50% CPE and 50% CNE, there was no statistical difference for any parameter compared to 60% CPE and 40% CNE and differed only for "taste" for 70% CPE and 30% CNE. The higher the concentration of coconut extract, the higher its ratings for all attributes. Regarding the "overall impression" and "color" attributes, the sample with 100% CPE was significantly different from the others, which goes according to the results in color analysis. In respect to taste, the sample containing 50% CNE and 50% CPE attained the best scores. However, it did not differ significantly from the extract containing 60% CPE and 40% CNE. 100% CPE had the lowest score, but with no statistical difference to 90% CPE and 10% CNE.

The low scores may be justified by the fact that no other ingredient, such as sugar, coffee, or chocolate, has been added to the developed beverage. It is a common practice of people who consume cow's milk as well as people who drink plant-based milk (Pineli et al., 2015).

Analyzing the acceptance percentages of the first step of sensory analysis, none of the six samples reached the acceptance scale. That is, for all parameters, none reached more than 70% (Minim, 2013, p. 332). Thus, the sensory characteristics of the developed plant-based milk must be improved, such as the addition of flavoring. Therefore, a second sensory evaluation was conducted.

Regarding the participants of the research, 40% reported that they rarely consumed some of the plant-based milk, and 13% declared never to drink this type of beverage. Only 28% of the participants had frequent (at least biweekly) consumption of plant-based milk. When asked about their acceptability of these products, 20% scored on the rejection scale. This result may have interfered with the low scores in the sensory analysis performed since it was not conducted with the target public.

Results point out higher scores for the plant-based milk made from chickpea and coconut when compared to the scores obtained in the study of Makinen et al. (2015). Regarding the "overall impression" attribute, Makinen et al. (2015) found 4.9 for soymilk, 4.7 for oat extract, 3.2 for quinoa extract, and 4.5 for rice extract, using a 9-point hedonic scale. Another study by Pineli et al. (2015) developed quinoa-based milk and compared it to industrialized rice extract, also using a 9-point hedonic scale. They did not get good scores: 4.4 in the attribute: "overall impression" for the developed beverage and 4.6 for the rice extract. All these scores were lower than the scores found for the beverages developed in this study with the addition of coconut extract.

The second step of the sensory analysis was performed with 28 participants, mean age 29 years old, 71% female. In addition to the inclusion criteria defined in the first analysis, these panelists also had to be frequent consumers of plant-based milk (biweekly or more) and/or like this type of product (like slightly or more). For this step, two of the developed samples were chosen, the sample containing 90% CPE and 10% CNE, and the sample containing 70% CPE and 30% CNE. The purpose of this analysis was to evaluate the sensorial attributes of the samples considering the lowest and highest coconut extract proportion, without statistical protein content differences. Vanilla extract (VE) in the concentration of 0.3% was added to the samples to investigate whether flavoring could improve the sensory attributes. Thus, panelists evaluated four samples, two without vanilla (90% CPE and 10% CNE; 70% CPE and 30% CNE) and two with vanilla (90% CPE and 10% CNE + VE; 70% CPE and 30% CNE + VE). The results are described in Table 4.

Results from the second sensory analysis point out that the selection of specific consumers of the product had an impact on the ratings. The scores were notoriously higher when compared to the first sensorial analysis, which was performed by general assessors. Sample with 70% CPE and 30% CNE $\,+$ VE reached the acceptance scale for all attributes with scores equal or higher than 6.

Each pair of samples was analyzed separately, i. e., 90% CPE, and 10% CNE with and without vanilla and, 70% CPE and 30% CNE with and without vanilla. It justifies the use of the t-test in statistical analysis for this part. For the "overall impression," there was no statistical difference between the scores for the two pairs. That is, the addition of vanilla extract did not differ for this parameter.

When comparing the first pair of samples, a significant difference was noticed in the attributes "odor" and "taste." That is, the flavoring of

the beverage in this concentration improved the sensory aspects. In the study by Pineli et al. (2015), the authors suggest flavoring the developed quinoa extract to investigate the acceptance among potential consumers. This strategy was tested in the present study, and results showed that flavoring improved taste and odor.

For 70% CPE and 30% CNE, there was no statistical difference for any attribute except "odor." That is, for higher concentrations of coconut extract, the panelists of the study could not perceive the difference of the samples with and without vanilla regarding taste. Thus, there is no need to flavor the developed beverage with higher concentrations of coconut extract, since coconut is already an excellent flavoring ingredient.

When the acceptance percentage is analyzed, the sample with 70% CPE and 30% CNE \pm VE reached 71% of acceptance for the attribute "overall impression," and 71% for "taste." Thus, the 70% CPE and 30% CNE \pm VE stood out in comparison to the others.

This study presented limitations. The shelf life of the samples was not evaluated to give consumers the number of days they can be fridge stored. Also, samples were not pasteurized or sterilized as cow's milk is to be commercialized. Changes in color and taste can occur.

4. Conclusion

Novel plant-based milk based on chickpea and coconut was developed in this study. The beverage displayed a good nutritional composition (such as protein, calcium and lipid content) when compared to cow's milk and other common substitutes for cow's milk, such as oat, almond, and rice beverages. Those alternatives contain very low protein content, while the developed plant-based milk reached higher protein and calcium values.

Acceptance of the developed plant-based milk was low in the first sensory evaluation but presented better assessment than other plant-based beverages described by other authors. When plant-based milk customers evaluated chickpea and coconut samples, and it was flavored with vanilla extract, better acceptance was reached with levels higher than 70% of acceptance for 70% CPE and 30% CNE. It is essential to clarify that the samples were offered without any other added ingredients, such as sugar or chocolate.

Plant-based milk based on chickpea and coconut (formulation with 70% CPE and 30% CNE plus 0.3% vanilla extract) may be a potential substitute for cow's milk, considering the nutritional quality aspects (mainly protein and calcium content), acceptance and low allergenicity. Thus, future studies should be conducted to test this beverage with the inclusion of technological processes, such as microbiological analysis, homogenization, and pasteurization for large-scale production.

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CRediT authorship contribution statement

Luana Rincon: Conceptualization, Investigation, Resources, Writing - original draft, Visualization. Raquel Braz Assunção Botelho: Conceptualization, Methodology, Writing - review & editing, Supervision, Project administration. Ernandes Rodrigues de Alencar: Methodology, Formal analysis, Writing - review & editing, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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