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Optimization and consumer acceptability of carob powder as cocoa substitute in lactose-free cashew nut almonds-based beverage

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Abstract

Several benefits are associated with cocoa powder consumption as antioxidant properties and cardio protective effect. However, due to some disadvantages, such as the presence of stimulating and allergenic substances, products have been developed using carob powder as cocoa substitute. The product does not have the same disadvantages as the cocoa, besides been naturally sweetened and is a good source of fiber. The objective of this research was to develop a lactose-free cashew nut almond (CNA) beverage similar to a chocolate milk beverage and to optimize the beverage by estimating the feasible percentage of substitution of cocoa for carob powder and the ideal sucrose and carrageenan content. In the first phase, a 2^3 factorial planning type was carried out. As for the second phase, seven samples were subjected to acceptability tests carried out with 70 untrained panelists. By response surface methodology, the total substitution of cocoa for carob powder (2 g/100 mL) in lactose-free CNA-based beverage is feasible. The seven formulations achieved good sensory acceptance. The concentration of sucrose exerts a great influence on the sensory acceptance of the beverage. It is recommended the use of 18.8% sucrose.

Introduction

The cashew tree (*Anacardium occidentale* L.) and its fruit, cashew nuts, are a key agent of the brazilian export industry. Quality standards exist to measure integrity, color, size and flavour set by the Association of Food industries, which influences the average export price. As 40-45% of the almonds are broken during processing, leading to their rejection and the loss of 27% in the average export price it is important that uses are identified for broken CNA to increase its value for the industry and reduce net loss (Pinheiro *et al*., 2006; USAID, 2006; Parreiras, 2007; Moura and Magalhães, 2008).

Therefore it is important to develop new products with broken CNA to add value to them. Morais and Rodrigues (2015) developed a beverage alternative to cow milk using CNA. The beverage could be consumed especially by people with lactose intolerance. This condition is very common to adults worldwide. People with lactose intolerance may present or not severe symptoms only by consuming a glass of milk, according to the degree of lactose intolerance. Some symptoms are abdominal bloating and pain, flatulence, diarrhea, gas, nausea and vomiting. Avoid the ingestion of dairy products by people diagnosed with lactose intolerance leads to the disappearance of symptoms (Lovelace and Barr, 2005; Alliende, 2007; Law *et al*., 2010).

Cocoa powder is widely used in the formulations of chocolate milk products along with sugar, aroma and other ingredients, so it could also be applied in the beverage similar to milk, obtained from the CNA of lower commercial value (broken ones), to obtain a product similar to a chocolate milk beverage. Some benefits to human health are associated with the consumption of beverages or products containing cocoa, due to the presence of phenolic compounds, such as antioxidant (anti-carcinogenic) and cardio-protector (Nehlig, 2013; Ali *et al*., 2015; Grassi *et al*., 2015).

However, because of some disadvantages, such as low solubility, bitterness, high content of saturated fat and the presence of stimulating and allergenic substances (phenylethylamine), several products have been tested and applied as cocoa substitutes. Among the possible substitutes, cocoa and chocolate aromas, cupuaçu powder and carob powder stand out (Lannes *et al*., 2002; USDA, 2014; Rosa *et al*., 2015).

Considering that the CNA-based beverage is an alternative proposal for people with feeding restrictions, it is relevant to study the use of carob powder due to the fact that it is a natural product that has been tested in the formulations of several foods

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and beverages as a cocoa powder substitute (Barroso *et al*., 2015; Rosa *et al*., 2015; Srour *et al*., 2016).

Carob powder is naturally sweetened and require less industrialized sugar addition in the products in which it is added. It is similar to chocolate in appearance being obtained through the roasting and grinding of pods and predominantly contains sugar and fibers, having insignificant lipid content (Yousif and Alghzawi, 2000; Jambi, 2015). It does not have stimulating compounds and the cocoa's characteristic bitterness. Moreover, it has good solubility (Yousif and Alghzawi, 2000; USDA, 2014). Carob powder is a ingredient with a considerable nutritional value due to its high dietary fiber and phenol compounds. The soluble fibers are thought to exert a preventative role against heart disease and lowering serum cholesterol while the polyphenols have antioxidant activity and are involved in protection against several diseases as cardiovascular and neuronal. Thus, Carob powder is an ingredient that can be used in functional and healthy foods (Wang *et al*., 2002; Owen *et al.,* 2003; Youssef *et al*., 2013).

The main objective of this paper was to develop a lactose-free cashew nut almond (CNA) beverage similar to a chocolate milk beverage and to optimize the beverage by estimating the feasible percentage of substitution of cocoa for carob powder and the ideal sucrose and carrageenan content.

Materials and Methods

Samples composition and preparation

The samples were prepared using processed CNA, classified as B1 (type 1 butts; Assn. of Food Industries 2012), and mineral water (Indaiá, Fortaleza, Brazil). The CNA were initially sanitized with commercial hypochlorite solution Pury Vitta with 0.96% w/w active chlorine concentration (Meneghetti Indústria Química Ltda., Dois Córregos, Brazil) and weighed according to the proportion 1:6 (CNA: mineral water). The CNA-based beverage was produced by soaking and grinding almonds. The extraction was carried out in the Soya Milk Machine 500-2 (Soya Milk Machine®, Sorocaba, Brazil). Then, the CNA were crushed with mineral water in the machine. The process occurs over 24 minutes with gradual warming to 95 C and pauses in crushing. Then the bevegare was filtered through a 30 mesh sieve for separation of the starchy residue (wet flour). Add sucrose (União, São Paulo, Brazil), κ-carrageenan (Doce Aroma Aditivos e Ingredientes, Tatuapé, Brazil), cocoa powder (Mãe Terra, Brazil) and/or carob powder (Amendoim & cia, Araquari, Brazil) to the filtrate according with the formulation and homogenized for three minutes at low speed. The beverage was packed in polyethylene bottles, subjected to pasteurization at 72 C for 20 min in a water bath, followed by cooling to 4°C on ice and water bath. The product was stored under refrigeration (5°C ± 2°C).

Experimental factorial design

The application of the experimental design aimed to estimate the feasible percentage of substitution of cocoa for carob powder in a 2 g/100 mL concentration and the sucrose and carrageenan concentrations. As carob has natural sugars, TSS (total soluble solids) were assessed to the decision on the percentage of substitution.

A full factorial design (2^3) was applied to investigate the influence of the ratio cocoa / carob powder (2 g /100ml) and of the sucrose and carrageenan concentrations in TSS of the CNA-based beverage with cocoa and / or carob. It was employed a central composite rotatable design –CCRD (Box and Wilson 1951, Box and Hunter 1957), second order model (Table 1).

The design was generated using the R software (Kuhnert and Venables 2005), where the order of the points was randomized by the program and the experiments were carried out in this order. The limits are established in according with cocoa, sucrose and carrageenan concentrations used by Yanes *et al.* (2002), Prakash *et al*. (2010) and Tarrega *et al*. (2012) in formulations of chocolate milk beverage.

The measurement of TSS (°Brix) was performed in duplicate with portable digital refractometer, measuring range from 0.0 to 53. ° Brix, PAL-1 model, Atago (ALI, 2004).

Sensory evaluation

In the second phase of the optimization, besides the formulation optimized with 100% of carob powder (2 g /100mL), samples with intermediate ratios of cocoa/carob were also evaluated in order to verify alterations caused along the gradual substitution.

The formulations were defined based on existing combinations in the experimental design (Table 1), selecting those presenting a greater comprehensiveness regarding the ratio cocoa/carob and the concentrations of sucrose and carrageenan. The samples were designated as LSuB, HCarB, HSuB, HCabB, CoB, CabB, LCarB, respectively (Table 2).

The sensory analysis was carried out with 70 untrained panelists, the majority of whom were women (60%), aged between 18 and 30 (77.4%), undergraduate students or graduated (84.3%). Panelists were selected based on their regular

Table 1. Experimental design for preparation of CNA-based beverage with carob and/or cocoa powder and TSS results.

Formulation	Independents variables - Uncoded and coded values			Dependent variables
	S (%)	Ca (%)	Co (%)*	TSS (°Brix)
F1	13 (0)	0.0650 (0)	50 (0)	15.8
F2	8 (-1)	0.0300 (-1)	79.7 (1)	14.6
F3	18 (1)	0.1000 (1)	20.3 (-1)	15.5
F4	13 (0)	0.0650 (0)	50 (0)	15.7
F5	13 (0)	0.0650 (0)	50 (0)	15.9
F6	8 (-1)	0.1000 (1)	79.7 (1)	17.7
F7	13 (0)	0.0650 (0)	50 (0)	14.6
F8	18 (1)	0.0300 (-1)	79.7 (1)	18.1
F9	8 (-1)	0.0300 (-1)	20.3 (-1)	13.8
F10	8 (-1)	0.1000 (1)	20.3 (-1)	14.4
F11	18 (1)	0.0300 (-1)	20.3 (-1)	17.6
F12	18 (1)	0.1000 (1)	79.7 (1)	16.5
F13	13 (0)	0.0650 (0)	50 (0)	15.5
F14	13 (0)	0.0650 (0)	50 (0)	15.6
F15	13 (0)	0.0650 (0)	50 (0)	15.5
F16	13 (0)	0.0061 (-1.6818)	50 (0)	15.8
F17	13 (0)	0.0650 (0)	99.95 (1.6818)	16.1
F18	13 (0)	0.0650 (0)	50 (0)	16.9
F19	13 (0)	0.0650 (0)	50 (0)	15.5
F20	13 (0)	0.0650 (0)	0.05 (-1.6818)	15.8
F21	13 (0)	0.0650 (0)	50 (0)	15.9
F22	4.59 (-1.6818)	0.0650 (0)	50 (0)	7.5
F23	13 (0)	0.1239 (1.6818)	50 (0)	14.7
F24	21.41 (1.6818)	0.065 (0)	50 (0)	19.9

Su=sucrose, Ca=carrageenan, Co=cocoa. TSS=total soluble solids (°Brix).
*In relation to a concentration of 2 g/100 mL with complement of carob powder.

Table 2. Formulations of lactose-free CNA-based beverage with cocoa and / or carob powder analyzed in the sensory evaluation.

Samples	Ingredient (%w/v)			
	Sucrose	Carrageenan	Cocoa powder*	Carob powder*
LSuB	4.59	0.065	50.00	50.00
HCarB	18.00	0.10	79.70	20.30
HSuB	21.41	0.065	50.00	50.00
HCabB	8.00	0.03	20.30	79.70
CoB	13.00	0.065	100.00	0.00
CabB	13.00	0.065	0.00	100.00
LCarB	13.00	0.0061	50.00	50.00

L=low, H=high, Su=sucrose, Car=carrageenan, Cab=carob, Co=cocoa, B=beverage.
*In relation to a concentration of 2 g/100 mL.

consumption of CNA and of beverages alternative to milk. The acceptability tests were conducted under white light, in individual and ventilated tasting booths in Sensory Analysis Laboratory. The design was balanced complete blocks and the samples were presented monodically in two sessions. The samples (30 mL) were served at 10°C ± 1°C in transparent acrylic glass coded with random three-digit numbers. Mineral water at room temperature (20°C ± 2°C) was provided for mouth-rinsing (Vickers 1988; Stone and Sidel 2004). The 9-point hedonic scale (1 = dislike extremely, 5 = neither like or dislike, 9 = like extremely) was used to assess the overall acceptance, according to Stone and Sidel (2004). The appropriate sweetness intensity was evaluated using a 7-point JAR scale, where 1= much too weak, 4= just about right and 7= much too strong (Meilgaard et al., 2007).

The study was reviewed and approved by the Federal University of Ceará's institutional ethics committee (21335213.0.0000.5054). All subjects participated voluntarily and allergic subjects were not invited to participate.

Data analysis

The response surface methodology (RSM) was used to estimate the feasible percentage of substitution of cocoa for carob powder in a 2 g/100mL concentration and if sucrose and carrageenan concentrations influenced the TSS (° Brix) of the beverage (Khury and Cornell, 1996; Myers and Montgomery, 2002). The relationship between the independent variables was established through mathematical models. The model used was a second-order equation (1), where Y is the dependent variable (TSS), β_0 is the intercept (constant), X_1, X_2 and X_3 are the independent variables (sucrose, carrageenan and cocoa/carob), and β_1, β_2, β_3, β_{11}, β_{22}, β_{33}, β_{12}, β_{13} and β_{23} are the regression coefficients (linear, quadratic, and interaction). Data were also submitted to two-way ANOVA with interaction to identify significant linear and quadratic effects and of interaction at $p \leq 0.05$.

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \text{Error} \quad (1)$$

The principal component analysis (PCA) was applied to the overall acceptance data to obtain the loadings and assess panelists' preferences regarding the samples (Table 2). The analysis was performed using Statistica software version 7.0 (Statsoft inc., 2004). The hierarchical clustering analysis (HCA) was performed using XLSTAT Software (XLSTAT, 2008) to categorize the beverage's samples based on consumer's acceptance. The means of the scores obtained on the test using the 7-JAR scale were used to plot a graph depicting the sucrose concentration of each sample, and the exact amount of sucrose to be added to the formula with cocoa and / or carob powder to provide the ideal sweetness was estimated using linear regression – the point where y = 0 corresponds to the ideal concentration of sucrose (Vickers, 1988).

Results and discussion

Experimental factorial design

In the evaluation of the content of TSS of the beverage, the concentration of carrageenan and the ratio cocoa / carob, in a total concentration of 2 g/100 mL, does not affect ($p > 0.05$) the total soluble solids (Figure 1). Only the sucrose concentration had a significant effect ($p < 0.05$), with a significant increase in TSS with sucrose concentrations above 18%. There

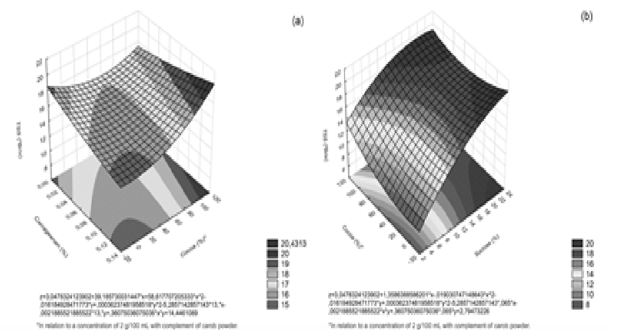

Figure 1. Response surface with the effects of (a) cocoa/ carob and carrageenan and (b) cocoa/carob and sucrose on TSS of the CNA-based beverage.
*In relation to a concentration of 2 g/100 mL with complement of carob powder.

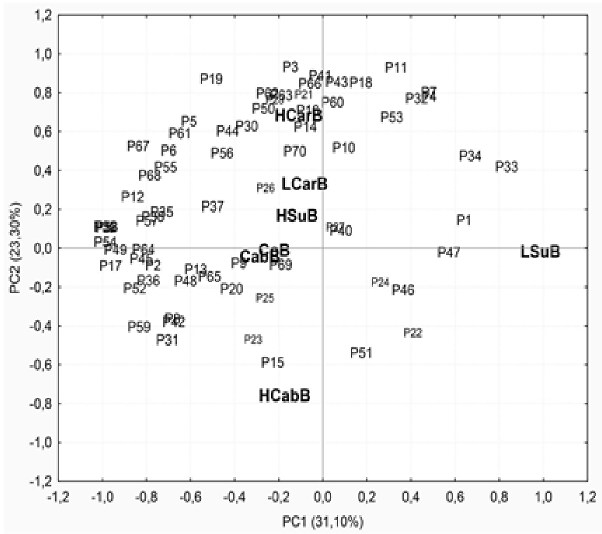

Figure 2. PCA loadings for panelists and CNA-based beverage samples with cocoa and / or carob powder.
See samples formulation in Table 2. P=panelist. PCA=principal component analysis. Panelists indicated in numbers and samples in bold letters.

was no interaction effect. This is to be expected, given that the soluble solids in a solution are usually related to the sugars present in the liquid. Even though carob powder have a considerable content of natural sugars in its composition (Yousif and Alghzawi, 2000; Owen *et al*., 2003; USDA, 2014), there has not been any influence ($p > 0.05$) on TSS. Probably, this behavior is due to the concentration used (2 g/100 mL). One also verifies that, the maximum region (darker) is situated above 100% cocoa (Figure 1), indicating that a concentration higher than 2 g/100 mL would have interfered in the TSS.

The present research aimed to test a total substitution. However, there was concern regarding the beverage's strangeness and the potential rejection of the product in sensory tests. Changes in TSS due to the substitution of cocoa for carob powder were unwanted as they could result in differences in the perception of the beverage's thickness or sweetness. Thus, as there was no significant increase in TSS in the beverage resulting from the total substitution of cocoa for carob powder, it was decided to recommend the total substitution of cocoa for carob powder. Nevertheless, for the final definition of the beverage formulation, a second phase of the optimization was carried out, in which the acceptability of the product was evaluated according to formulation variation.

The selected formulations presented a greater comprehensiveness regarding the ratio cocoa / carob and the concentrations of sucrose and carrageenan. Thus, formulations 22, 12, 24, 9, 17, 20 and 16 were used for the samples designated as LSuB, HCarB, HSuB, HCabB, CoB, CabB, LCarB, respectively. Formulations 22 and 24 were chosen for presenting sucrose concentration extremes and formulations 12 and 16 for presenting carrageenan concentration extremes. Formulations 17 and 20 presented almost 100% cocoa and 100% carob, where, in the second phase of the optimization, the values were adjusted to 100% (2 g/100mL). And formulation 9 was also chosen for having cocoa/carob, sucrose and carrageenan intermediate values, totalizing seven samples to be analyzed in the second phase of the research.

The acceptance test was not carried out using an experimental design due to the large number of formulations that would have to be evaluated by panelists (n=24). The recommended number of products that panelists should evaluate by session is limited to five or six, or fewer, depending on the type of product and on the possibility of causing sensory fatigue. More products could be evaluated; however, it would require participants returning for other sessions either later that day or on another day. Nevertheless, it is difficult to guarantee that the panelists will return when many sessions are required. An alternative approach for tests of larger numbers of products is the use of incomplete designs; however, this approach requires an increase in the number of subjects, which is also a limiting factor (Stone and Sidel 2004).

Sensory evaluation

The segmentation of consumers concerning sensory acceptance of the evaluated samples can be viewed through the principal component analysis (PCA) score plot (Figure 2), where are indicated the panelist in numbers and the samples in letters. The proximity of the symbol indicates the preference for such sample.

Component 1 explained 31.10% of the variation in the acceptance of the seven CNA-based beverage samples with cocoa and/or carob, while component

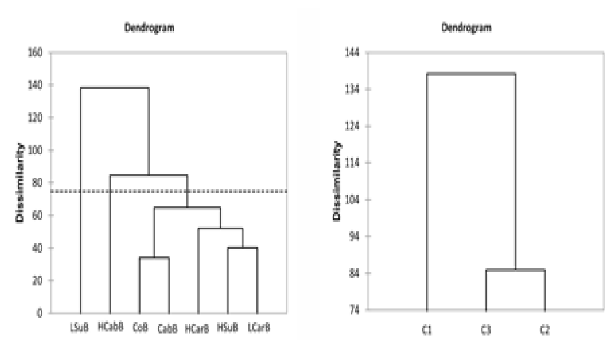

Figure 3. Dendrogram for the hierarchical cluster analysis (HCA) results according to the sensory acceptance for the samples of CNA-based beverage with carob and/or cocoa powder.
See samples formulation in Table 2.

2 explained 23.30%, comprehending 54.40% of the grand total.

The samples of CNA-based beverage with cocoa and / or carob powder were accepted. Srour *et al.* (2015) developed a carob-based milk beverage and achieved good acceptability ratings. A different result was obtained by Rosa e*t al.* (2015) when studying the effect of substituting cocoa for carob powder in gluten-free cakes. The authors found that acceptance decreased gradually with the substitution of cocoa for carob and that the product is no longer accepted with a substitution of over 75%. Aydin and Özdemir (2017) developed a carob powder-based functional spread for increasing use as nutritious snack for children and found that higher amounts of carob powder were not accepted.

It was noticed that the panelists preferred the HCarB, LCarB and HSuB samples, followed by the CabB and CoB samples, for most representative points are located close to such samples. The HCabB and LSuB samples were the less preferred, having the LSuB sample been the least preferred one. It is worth highlighting that, the LSuB and HCabB samples had the lowest sucrose concentrations; 4.56 and 8.0%, respectively, whereas the preferred samples had sucrose concentrations between 13 and 21.41%.

The HCA results confirm and complement the information obtained by the PCA. The beverage samples were grouped in the HCA graphical presentation (Figure 3). The dendrogram demonstrates that the samples were clustered into three classes according to the samples' sensory acceptance. The first and third classes consist of only one sample each: LSuB and HCabB, respectively. The second class consists of all other samples, indicating that there is no preference for any of them. The groups formed based on HCA results confirmed that the proportion of substitution of cocoa for carob powder does not influence the overall acceptance of the beverage. In fact, sucrose concentration is what influences acceptance. The product had a reduced sensory acceptance with low concentrations, i.e., formulations with lower percentages of sucrose (LSuB and HCabB) were less accepted. In addition, these samples were separated into distinct classes according to the variation in sucrose concentration of less than 4%. The samples grouped in the second class exhibited an 8% variation in sucrose.

Generally, the ratio cocoa / carob and the carrageenan concentration do not exert great influence over the beverage acceptance, being relevant the sucrose concentration. One observes that sucrose concentration between 13 and 21.41% result in greater acceptance.

From the equation of the straight line obtained (2), it was calculated that the concentration of sucrose to be added to the CNA-based beverage with carob and / or cocoa powder is 18.8% (R^2=0,9818). It should be noted that 18% is the value from which a significant increase in TSS occurs, as depicted in Figure 1, thus demonstrating that higher concentrations lead to a sensory perception of the increase in sweetness.

$$y = 14{,}541x - 2{,}7342 \qquad (2)$$

The JAR scale has been used to define the ideal intensity of sweetness. The optimal concentration of sucrose (%) to be added to various beverages was determined using JAR scale and linear regression by Cardoso *et al*. (2004), Moraes and Bolini (2010) and Villegas *et al.* (2010).

Conclusion

The CNA-based beverage is well accepted both with cocoa and carob powder, thus indicating that the total substitution of cocoa for carob powder is totally feasible. The ratio cocoa / carob powder (2 g/100 mL) seemed not to affect the beverage's sensory acceptance, while sucrose concentration exerted great influence. It is recommended 18.8% of sucrose to obtain the ideal sweetness in the CNA-based beverage with cocoa and / or carob.

Carrageenan can be used in the concentration of 0.1% to ensure greater stability of the beverage. Thusly, the CNA-based beverage with cocoa and / or carob powder can constitute as alternative to the consumption of chocolate milk beverages by people with problems related to milk ingestion, like lactose intolerance. It is recommended the total substitution of cocoa powder for carob powder. This way it would also be an option for individual those who do not want to ingest stimulating and allergenic substances or just wish to enjoy the benefits of carob as the natural sugars, fibers, phenol compounds, insignificant lipid content and absence of bitterness.

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