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Nectar Mix Functional Based on Amazonian Fruits

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Abstract

Background: Functional foods are characterized by offering several health benefits beyond the nutritional value inherent in their chemical composition. Fruits are good suppliers of minerals, vitamins, fiber, and water. Some fruits can even provide small amounts of carbohydrates among other ingredients. Several fruit plants in the Amazon region have great economic potential due to their nutritious value. Fruits that are rich sources of dietary fiber, vitamin C and mineral elements. Implementation of Amazonian fruits cupuaçu (*Theobroma grandiflorum*), camu-camu (*Dunal Myrciaria dubia McVaugh*), cubiu (*Solanum sessiliflorum Dunal*), adjusting the proportions of the objectives of the functional product of biotechnology, aimed at producing a product with low energy and high nutritional value.

Subjects/Methods: The preparation consisted of chemical and physicochemical analyses composition, analysis of inorganic constituents of the fruits, product formulation, microbiological analysis of formulated products, and sensory analysis. The sensory analysis of nectar was divided into nine sets, where for each concentration of pulp was determined the best concentration of sucralose. The experimental design used the factor 33 and descriptive statistics.

Results: We developed a low calorie bioactive dietary nectar mix, sweetened with sweetener sucralose. The microbiological analysis of the formulation showed: Coliform bacteria, 0/ml; mesophylls (CPP) <10UFC/ml; psychrophilic (CPP) <10UFC/ml, molds and yeasts <10UFC/ml, and some *Salmonella sp*. The result was a low-energy product, 59.441 kj/100 ml, and the nutritional assessment shows: Potassium 103.23 mg/100 ml; Calcium 7.15 mg/100 ml; Phosphorus 7.30 mg/100 ml; Fibers 1.45 g/100 ml; Vitamin B_3 0.43 mg/100 ml; Vitamin C 260.83 mg/100 ml. The experimental design used the factor 3^3 and descriptive statistics showed confidence interval 95% and P value 0.03 and 0.58.

Conclusion: This product can be consumed by almost all the population assisting in the nutrition of children, youth, adults, elders, and even during pregnancy. It can also to be assigned of diet and still be recommended to individuals with restriction of sugars.

Keywords: cupuaçu (*Theobroma grandiflorum*); camu-camu (*Myrciaria dubia Dunal Mcvaugh*); cubiu (*Solanum sessiliflorum Dunal*); sucralose

Introduction

Amazonian fruit trees are of great economic potential for its fruits are rich source of fibers and countless nutritional benefits. A variety of native tropical fruits can be easily found at riverbanks, lakes, and Amazonian floodplains. Camu-camu stands out for its high content of vitamin C (30 times higher than the Orange fruit), which normally have 2800 mg/100 g of the fruit, and could be reached even more than 6.000 mg/100 g against the average of 1700 mg/100 g of *Acerola* fruit [1].

Cupuaçu (*Theobroma grandiflorum*) is a tropical fruit that can be easily found around rivers, and lakes in the south and northeast of *Maranhão* and neighboring areas. *Cupuaçu* has a whitish citric pulp with a nice and strong aroma. Local folks use its pulp to make cakes, cookies, candy, mousses, puddings, pie, ice cream, jelly, jams, among other pastries applications. *Cupuaçu* has one of the best industrial application benefits due to its variety of food applications and high nutritional level [2].

Cubiu (*Solanum Sessiliflorum*) represents another exotic example of Amazonian fruits popularly known as Indian tomato and *maná* [3]. It contains high level of Niacin [4,5] and Iron [6]. It can also be utilized in *natura* or ingested in the ways of juices, sweets, jam, jelly, ice-cream, sauce, cosmetic products [7], as well as in popular Medicine due to their hypoglecemy and hypocolesterolemic agents [8,9].

Preliminaries studies of eight varieties of *Cubiu* fruits have showed that it has a low energetic level (average of 138.138 kj) and the fiber presented has a total of 1.6%. It's an eccentric and has proper characteristic flavor, allowing it to be used in many ways: juices, jelly, jams, sauce, as spices for beef, pork, sheep, and also used for the cosmetic products [1].

Studies done in rats indicate the existence of hypoglycemic substance in the Cubiu [10] confirming its traditional popular medicine utilization to control high levels of cholesterol, uric acid and sugar in the blood [10].

Camu-camu (*Myrciaria dúbia Dunal McVaugh*) is found at riverbanks, lakes and Amazonian lowlands. It is an excellent source of ascorbic acid, having high contents of vitamin C and great nutritional value [1]. Researches have revealed that Camu-camu outranks all fruits in terms of vitamin C concentrations [11].

Camu-camu is a dietetic fruit because it contains low levels of energy and abundant concentrations of food fiber. Vegetal food with iron content can have its absorption favored when it is ingested in conjunction with vitamin C. This practice can reduce the occurrence of anemia at local schools. *Camu-camu* is a very sour fruit. Therefore, it is rarely consumed in its natural form. In the other hand, *Cupuaçu* is consumed frequently.

Even though *Camu-camu* has great nutritional value, it is practically ignored by the population of the region like *caboclos*, who gives to it, only the use of fish bait.

Therefore, despite the discovery and disclosure of nutritional importance and the high concentration of vitamin C (2.891 mg) in the *Camu-camu* [11] , the great concentration of Calcium (23 mg), vitamin A (30 mg) and fiber (0.5 g) [2] in the Cupuaçu and the good concentration of Potassium (513.5 ± 3.1 mg), Calcium (18.9 ± 0.6 mg) [2] , Fe (564.4 ± 58,1 ug), Cr (99.3 ± 8.3 µg), K (229 ± 4.5 mg), Na (53.7 ± 5.5 µg) and Zn (89.3 ± 4.7 µg) [12] in Cubiu, those fruits are not commonly found at the table of Amazonian population [12].

Sucralose is an edulcorant derived from sucrose evaluated by the Food and Drugs Administration of the United States of America (FDA) [13] , European Food Safety Authority (EFSA) [14] , Health Canada, Australia and New Zeland Food Authority (ANZFA), Japanese Health Food, Joint FAO/WHO Expert Committee on Food Additives [15] (JECFA) formed by Food and Agriculture United Organization of the United Nations (FAO) [15] and World Health Organization of the United Nations (WHO). All these regulatory organizations concluded that the use of sucralose is safe as food ingredient by the general public, including children, pregnant and lactating women. Actually, it is safe to be used for people with diabetes for it does not affect the glucose or insulin blood levels. For this reason, sucralose is considered the only totally safe sweetener.

This study aimed to develop a low energetic and high nutritional value mix bioactive nectar from the fruits *Cupuaçu (Theobroma grandiflorum), Camu-camu (Myrciaria dúbia Dunal Mcvaugh) and Cubiu (Solanum sessiliflorum Dunal)* sweetened with sucralose, positively influencing in the reduction of overweight or obesity without any consumption restriction by the general population.

Material and Methods

The fruit *Cupuaçu (Theobroma grandiflorum)* utilized on this work was bought at a local open market (Mercado do Produtor) in the city of Manaus (Coroado neighborhood), and Rio Preto da Eva Municipality. It was submitted to a rigorous selection process in order to get the best readiness and sanitary acceptability. The fruit was packed in polypropylene bags and transported to the laboratory of Coordination of Research in Health Science – CPCS of the National Institute of Amazonian Researches.

The fruits were washed, sanitized and submitted to an analytical criteria processing before extracting its pulp. The fruits were washed by the running water and soaked for about 20 minutes. Afterwards, they were washed with a nylon brush of soft texture under running water until removing all dirt of the shell. During the sanitization process, the fruits were soaked for about 20 minutes in chlorinated water with residual chlorine free content of 100 ppm and washed in distillated water to remove all chlorine residues. At that point the fruit shells were broken over a stainless steel countertop using a stainless steel knife.

In the analytical criteria processing, with the fruit pulp exposed, it could identify any possible problems inside; the fruits that may show sign of fermentation, different coloration or signs of decay were discarded.

The pulp was manually removed and taken to the depulped for separating lumps, liquefied, and wrapped in polyethylene bags with a capacity of 500 g. It was immediately frozen and stored in freezers with temperature between -18°C and -22°C. The pulps were kept frozen until the time of use. This process can be seen in the Figure 1.

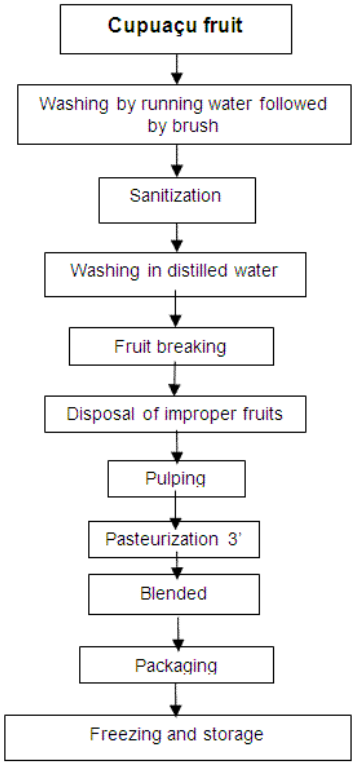

Figure 1: Processing stages of Cupuaçu fruit

The employed methods for the fruits processing are shown by the following subtopics.

The fruits of *Camu-camu (Myrciaria dubia Dunal Mcvaugh)* used in the research came from the Yurican Farm, localized at the kilometer 100 of the Road AM-010, in the municipality of Rio Preto da Eva. Collected ripe, selected according to the presence of mechanical injuries and sanity, washed in running water, immersed in a solution of sodium hypochlorite at 400 ppm, washed again and dried at room temperature.

After washing, the fruits were bleached at 70°C for 2 minutes to turn the oxidative enzymes inactive. Then, the fruits were pulped in stainless steel depulped, 1.5 mm grid, Itametal brand, at the nutrition laboratory of the INPA, taking all hygiene and sanitation care.

The pulps were packed in plastic packaging of Tupperware brand with capacity of 500 g and hermetic closure and selected according to the criterion of adequacy for food products. The pulps were packed and stored as can be seen in the Figure 2. The pulps were immediately frozen at temperature of -18° C until the moment of its utilization.

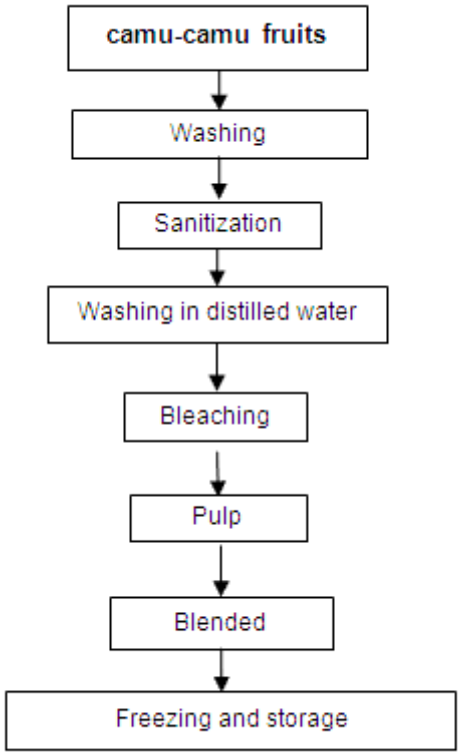

Figure 2: Processing stages of fruit camu-camu

The fruits of *Cubiu (Solanum sessiliflorum Dunal)* used in this research came from the Experimental Station of the National Institute of Amazonian Researches, localized at the kilometer 14 of the Road AM-010, in the municipality of Manaus.

The selection followed the same criteria as to sanity and maturity stage, described for the camu-camu fruit. The procedure for obtaining the pulp consisted in washing the fruit in running water and removing of peduncles, shell and placenta by the use of stainless steel knife. Regarding the cleaning process it was used the method of processing to obtain pulp, freezing and storage quoted for Camu-camu.

In Figure 3 are found the phases since from the arrival of the fruits, until stored in plastic bags and pots suitable for this purpose.

The pulps were totally defrosted and homogenized for testing the nectar formulation and for physicochemical analyses of moisture, lipid, protein, pH, total and reducing sugars, ascorbic acid, phenolic compounds, total anthocyanin and flavonoids according the methods recommended by AOAC [16].

The sweetener used for processing and obtaining formulated products in this work was sucralose in its pure form in insignificant quantities. This sweetener was imported from Finland and acquired from Tovani Benzaquen Com. Imp. Exp. and Representations, located in the State of São Paulo.

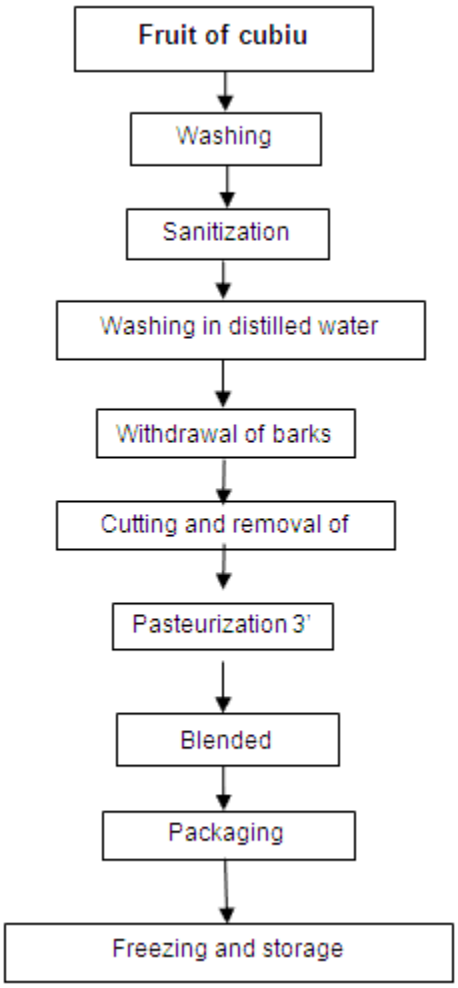

Figure 3: Processing stages of fruit Cubiu

The analytical reagents used for chemical, physical-chemical and microbiological analysis were acquired with the resources of the Project financed by the National Council for Scientific and Technological Development – CNPq. The equipment for these analyzes were provided by INPA as permanent material and other were purchased with the CNPq resources.

The ingredients used in the nectar formulation meet the requirements for microscopic testing according to the Resolution 12/78 from CNNPA/MS [17], and comply with the Articles 2nd and 3rd of the Normative Instruction nº12, dated of September 4, 2003 from the Legislation for Fruits and Juices [18]. Considering that the pulp of a particular fruit not fixed at a specific Technical Regulation should contain at least 30% (m/m) of the respective pulp; except in the case of fruit pulps with high acidity or high pulp content, or very strong

flavor. In this case, the pulp content should not be less than 20% (m/m).

The nectars were obtained by the mixing of pulps in accordance with the legislation [17], sucralose and water, followed by stirring until it turned homogeneous. In order to select the best formulation, 27 different concentrations of pulp and sucralose samples were tested. These samples were submitted to sensorial evaluation [19], reducing it to nine samples on a scale of preference and these to three samples of greater acceptability. For these selected samples were assigned other numbers of identification and submitted to a new trial with the same 30 tasters. The optimal formulation was chosen by sensory analysis employing preference test [19]. The tests were performed at the Laboratory of Nutrition/CPCS/INPS. All the different formulations of the nectar were served cold in temperature hovering between 7°C and 8°C for a group of 30 untrained tasters.

The results of the formulations were achieved through the use of factorial experimental design, an important application in combinatorial analysis. There were performed 21 combinations, of which 6 were discarded because they were made equal. When there is only one combination is because all the combinations are equal, we consider only a combination.

Results and Discussion

During the preparation of different formulations containing these three fruits and sucralose, it was observed the minimum percentage recommended by Normative Instruction nº12, dated September, 2003 from the Legislation for Fruits and Juices [18]. In the specific case of these fruits that are acidic, have too high pulp content and possess very strong taste, the pulp content should not be less than 20% (m/m). Hence, all formulations were above the minimum.

The preparation of formulations also attended the norms of Resolution RDC nº3, dated January 2001 [20], which determined the maximum use of 0.025 mg/100 ml for sucralose. However, during the study this resolution was modified by Ordinance nº354 by ANVISA, dated August, 2006 [21] that extends to the maximum limit to 0.075 mg of the sweeteners additive in 100 g or 100 ml of carbonated and non-carbonated soft drinks as ready to eat food. This alteration meets the references of the *Codex Alimentarius* [22] and the European Union [23] for the uses proposed. However, in the formulations were considered the use of sucralose under specific conditions and at the lowest level to achieve the desired effect.

After identifying the formulations with high acceptability, they were identified by numbers and were again subjected to the evaluation aiming to find the best choice.

A Microbiological Evaluation of nectars was done in triplicate samples for the selected formulations from best choices tested and the results can be seen in Table 1.

Ordinance No 451 of 19 September 1997 [24] establishes the microbiological standard toilets only for fecal coliforms and Salmonella, the maximum 104/ml for counting the yeasts and molds, for food and non-alcoholic beverages (soft drinks, juices and nectars, added to conservatives and ready to consumption) and regulates it to fresh juices and refreshments.

Sample	**Pasteurization 3 minutes**					
	MPN/mL	MPN/mL	CFU/mL	CFU/mL	CFU/mL	25 mL
	Total coliforms	Fecal coliforms	Psychrophilics (CPP)	Mesophilic (CPP)	Molds/ yeasts	*Salmonella sp*
1	0	0	<10CFU	<15X10^{1}CFU	10X10^{1}CFU	Absence
2	0	0	<10CFU	<10CFU	14X10^{1}CFU	Absence
3	0	0	<10CFU	<10CFU	<10CFU	Absence
* Averages of analyzes on three bottles randomly chosen in batch. MPN/mL (Most Probable Number per milliliter) CFU/mL (Colony Forming Units per milliliter) SPC (Standard Plate Counts)						

Table 1: Results of microbiology* applied to the analysis of three bottles of the batch

The aforementioned Ordinance [24] provides instructions regarding the interpretation and conclusions of the microbiological analysis of foods destined for human consumption taking as products in unsatisfactory hygienic conditions those who present standard plate count, total coliforms, yeasts and molds above the established limits and a maximum of ten (10) times these limits. Also determines that products in unsatisfactory hygienic-sanitary conditions are those which have fecal coliform, *Staphylococcus aureus, Bacillus cereus* and sulphites reducers *Clostridium* (at 46°C) above the limits of specific patterns and up to a maximum of ten (10) times these limits [24]. Considers as products potentially capable of causing disease ones which present *Staphylococcus aureus, Bacillus cereus, Clostridium perfringens* and its indicators (sulphites reducers *Clostridium* at 46°C) in a number more than ten (10) times the limits in specific patterns. Further included microorganisms infective such as: *Salmonella spp, Yersinia enterocolitica, Brucella spp, Campylobacter jejuni* and others recognized and characterized as agents of foodborne infections [24].

The results presented in Table 1, and when confronted with the established standards [24] show they are consistent with regulation of microbiological standards sanitary for food and non-alcoholic beverages (soft drinks, juices and nectars, added to conservatives and ready for consumption) [25]. This check reveals satisfactory hygienic

conditions, hygienic and sanitary conditions and potential inability to cause disease.

The Nutritional Evaluation of Nectar was performed using chemical analysis in triplicate to determine the chemical composition and the calculation of soluble and insoluble alimentary fiber was made by enzymatic-gravimetric method both according to the Association of Official Analytical Chemists - AOAC [26]. The fraction Nifext (carbohydrates) was obtained by the difference calculation of the other fractions analyzed. The total energy value was calculated from the sum of calories corresponding to proteins, lipids and carbohydrates (Nifext) which provide 20.511 and 16.144 kj/g respectively.

The determinations of mineral amounts were performed according to the method of Institute Adolfo Lutz [27] and with an atomic absorption spectrometer by flame SEPECTR AA - 10 PLUS (VARIAN). The quantification of the vitamin C was performed by titration with 2.6-dichlorophenolindophenol sodic [28]. All measurements were made using only the mesocarp of the fruits after bleaching and processing. It was found in *cubiu* (*Solanum sessiliflorum Dunal*) values of 4.50 mg/100 g, consistent with previous studies [28], and cupuaçu, 53 mg/100 g. In the camu-camu the value of vitamin C found was 2.585 mg/100 g. These values are superior to orange and acerola. The determined index of total polyphenol (TPI), total anthocyanins (TA) for cupuaçu was 20.5 ± 3.0. Moreover, camu-camu is a fruit highly dietetic by contain low energy and significant concentrations of dietary fiber.

In 100 ml of nectar mix we found the following results for Potassium: 103.23 mg/100 ml; Calcium: 7.15 mg/100 ml, Phosphorus: 7.30 mg/100 ml, Fiber: 1.45 g/100 ml, Vitamin B_3: 0.43 mg/100 ml and Vitamin C: 260.83 mg/100 ml

The results for Vitamin C and Potassium are greater than advised. The indicators suggest an intake of 45 mg/d of vitamin C for children under 13 and 75 mg/d for more than 13 years [28]. The recommendations show that the intake of Vitamin C must be 75 mg/d to 2000 mg/d for women and 90 mg/d to 2000 mg/d for men [28]. These values also apply to older adults over 70 years old and pregnant women. Regarding the potassium for adults the intake should be between 4.50 and 4.70 mg/d and for elderly over 70 years also is equal to 4.70 mg/d [28].

The elements found in the nectar mix and consumed in the daily diet can cause physiological benefits, due to the presence of these healthy physiological ingredients present in food, characterizing it like functional food [29]. These elements provide the opportunity to combine high flexibility of edible products with biological active molecules, as a strategy to consistently correct metabolic disorders, resulting in reduced risk of diseases such as overweight and obesity and development of health maintenance [30].

The sensory analysis [19] of nectar consisted of samples encoded using random letters and numbers. The visual, olfactory and gustatory sensory tests were performed with these formulations. In the visual test was evaluated the appearance and color of the product and in the olfactory sensory test parameter, acidity, sweetness and acceptability were evaluated. The tests were divided initially in nine independent from sensorial batteries, in which, for each concentration of pulp, the best concentration of sucralose was determined. The results allowed the identification of these preferred formulations of each battery, which led to three separate batteries, the result of which converged to the ultimate test, so that only the best formulation was selected at the end of the last test.

Tasters were asked to indicate with (X) the value that best described their impressions of each sample, using the scale below to describe how much they have liked or disliked. The evaluation of results was done by assigning grades. (1) greatly disgusted (2) very disgusted, (3) regularly disgusted, (4) slightly disgusted, (5) Indifferent, (6) I like it slightly, (7) good regularly, (8) I like it a lot, (9) good greatly. After that, we carried out a statistical analysis of responses to verify the acceptability of the product.

The results of the preference testes regarding the different concentrations of sucralose and pulp for each one are shown in Table 2, which showed greater preference for formulations 2, 3 and 9, set of batteries 1 and 3, which correspond to the concentration of 0, 02% of sucralose to two of the three formulations.

Formulações	Sum	Max	Min	Median	Average	SD	Variance	%
2	133,0	9,0	1,0	4,4	4,0	2,3	5,4	0,26
3	149,0	9,0	1,0	5,0	5,0	2,5	6,0	0,29
9	226,0	9,0	2,0	7,5	8,0	1,8	3,4	0,44

Table 2: Greater preference for formulations 2, 3 and 9, set of batteries analysis was performed on nectar mix formulation better accepted by the panel (9)

The physic-chemical analysis was performed on nectar mix formulation better accepted by the panel (9) (Table 3) at 20% and 60% respectively and are statistically described by the values of mean, variance, and confidence interval (p value)

The values are within the range expected for products based on fruit, it can be seen that pH has an average value of 3.27 ± 0.08 as characterized in the literature acidic (pH <7), and °Brix 13.4 ± 3.11, for which the legislation referred to when the liquid medium is composed of water or only part of the components of the fruit or juice product trout, and the concentration after the balance being no less than °Brix14.

pH 10	pH 12.5	median	Variance	*P*=value	°Brix 10	°Brix 12.5	Median	Variance	*P*=value

3.21	3.32	3.27 ± 0.08	0.01	0.03	11.2	15.6	13.4 ± 3.11	9.68	0.58

Table 3: Physicochemical analysis of the formulation nine (9) considered the ideal by the panelists

The formulation considered ideal by the tasters corresponds to a mix nectar functional characteristics with 59.441 kj/100 ml which may be administered in any population, considering that the present cubiu 171.626 kj /100 mg, cupuacu 301.392 kj /100 mg and camu-camu 71.162 kj /100 mg. The energy value of sucralose is neglected because it is not metabolized by the human body.

Foods that are specially formulated or processed, in which they introduce changes in nutrient content, suitable for use in diets, differentiated and / or optional, meeting the needs of people in specific metabolic and physiological conditions, according to the Ordinances SVS / MS 27 from 13/01/1998 [23] to and SVS / MS 29 13/01/1998 [24], Diet and Light are considered if they have low calorie (25% of standard).

According to the sensory evaluation [19], the nectar mix prepared showed acceptability to the 90.05% by the tasters, on a scale from 1 to 9 points. This result was higher than that obtained previously [31] that produced the camu-camu and obtained different levels of acceptance, on a scale from 1 to 9 points, representing 52-55% of acceptability.

There is no pattern for the preparation of mixed nectars, with fruits used in this study law. But Law no. 8.918/94 [32] specifies in Article 3rd that the nectar with a minimum amount of a particular fruit pulp has not been fixed at specific Technical Regulation should contain at least 30% (m/m) from its pulp, except for the case of fruit and acidity with very high content of pulp or very strong flavor and in this case, the content of the flesh should not be less than 20% (m/m). And in Article 13th further predicts that "the drink not provided in this Regulation may be disciplined by the Ministry of Agriculture and Supply, pursuant to the provisions concerning the classification and meet the characteristic of the product" [33].

The manufactured product meets the normality of the organoleptic characteristics of its own nature, quality and quantity of the components of its own nature, the absence of extraneous elements of indications of changes and pathogenic microorganisms, the absence of harmful substances and is complying with Regulation and legislation on additives.

Conclusions

Indigenous exotic Amazonian fruits cupuaçu, camu-camu and cubiu are characterized by their distinctive and characteristically tropical flavor that constitute this functional mix nectar [29,30] that for being a new product, it is not referred in literature on its physical-chemistry, but it shows that complies with the regulations of the sanitary microbiological standards for food and non-alcoholic beverages (soft drinks, juices and nectars added or not conservatives and ready for consumption) revealing it satisfactory hygienic and sanitary conditions which characterize as safe for human consumption [25].

Given the satisfactory assurance to the product as of low energy and high nutritional value and sweetened with sucralose hygienic and sanitary conditions can be given in any population, assisting in child nutrition, adolescent, adult, elderly and also the pregnant and still be designated as diet, may also be recommended to individuals with restriction of sugars, which characterizes it as safe for human consumption.

The elements found in the formulation considered ideal for this new product, under experimental conditions, confirm its characteristics of functional food and when consumed in daily diet can bring specific physiological benefits, thanks to the presence of physiologically healthy ingredients. The consumption that can be beneficial to health, contributing to an adequate diet is the maximum consumption values indicated by its components [28].

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