

Amazonian Fruits Antioxidant Capsules: Quality Control and Stability

Adele Salomão-Oliveira^{1*}, Grazielle da Costa Pontes², Kátia Solange Cardoso Rodrigues dos Santos³,
Emerson Silva Lima³, Rosany Piccolotto Carvalho⁴

¹Multi-Institutional Post-Graduate Biotechnology Program, Universidade Federal do Amazonas (UFAM), Manaus, AM, Brazil

²Department of Research, Society and Health, Instituto Nacional de Pesquisas da Amazônia (INPA), Manaus-AM, Brazil

³Pharmaceutical Sciences Department, Universidade Federal do Amazonas (UFAM), Manaus, AM, Brazil

⁴Physiological Science Department, Universidade Federal do Amazonas (UFAM), Manaus, AM, Brazil

*Corresponding author: adelesalomao@gmail.com

Received April 15, 2020; Revised May 17, 2020; Accepted May 24, 2020

Abstract The productive diversification in the Amazonian region is favored by the abundance and availability of its natural products and, biotechnology has stood out as an alternative in the innovative process of quality control and sustainable economic development. Based on this, one expects that bioproducts from this biome might favor the treatment of noncommunicable chronic diseases. The methods to be used for controlling the quality of the bioactive and microbiological compounds being encapsulated are operations necessary to guarantee the safety in the treatment with Amazon fruits consumed by individuals. Ascorbic acids and phenolic compounds analyses were carried out through HPLC and spectrometry, respectively. The present study has also ascertained the formulation to bear free radical scavenging activity in the DPPH tests. Thus, the methods used for controlling the quality of n.00 capsules and the Amazonian fruits formulation's bioactive content corresponded to the specifications included in the American Pharmacopoeia. Therefore, the differentiated quality control due to the large-scale production and the quantity of bioactive compounds originating from more than one Amazonian fruit points to the pharmaceutical specialties according to the international standards of quality of the health benefits to be made available to the society with the desired therapeutic effect, and characteristics appropriate for oral administration. The manipulated capsules were stored at 5 °C and the bioactive compounds stability was considered satisfactory in the preservation of their assets for 5 months with the produced capsules quality control guaranteed.

Keywords: *biotechnology, capsules, antioxidant stability, amazonian fruits, quality control*

Cite This Article: Adele Salomão-Oliveira, Grazielle da Costa Pontes, Kátia Solange Cardoso Rodrigues dos Santos, Emerson Silva Lima, and Rosany Piccolotto Carvalho, "Amazonian Fruits Antioxidant Capsules: Quality Control and Stability." *Journal of Food and Nutrition Research*, vol. 8, no. 4 (2020): 189-194. doi: 10.12691/jfnr-8-4-5.

1. Introduction

The chemical and nutritional characteristics of Amazonian fruits camu-camu (*Myrciaria dubia* (Kunth) McVaugh), açai (*E. precatória* Mart.) and guaraná (*Paullinia cupana*) arouse interest due to their high content of bioactive compounds and antioxidants, in addition to holding essential micronutrients, such as ascorbic acid, phenolic compounds and methylxanthines, which have protective effects on the development and progression of dyslipidemias and atherosclerosis, along with the importance of their impact on cardiovascular risk and other non-communicable chronic diseases (NCCD) [1,2,3].

Ascorbic acid is present with a relevant content in camu-camu, which belongs to the Myrtaceae family, and is involved in important metabolic and endocrine processes regarding the genesis and control of overweight,

becoming therefore fundamental as a factor of protection for dyslipidemias, which are related to the mortalities in Brazil [4]. It has shown to be associated with açai (*E. precatória* Mart.) and guaraná (*Paullinia cupana*), which have a high composition in bioactive compounds, important components for the health of the population due to their therapeutic biological activities [5].

Chronic diseases, mainly caused by inadequate lifestyle, were responsible for 2,813,503 million deaths in the United States in 2017 [6]. And it is expected that, in 2030, 72% of all deaths in Brazil will be due to these diseases, among which obesity stands out with an estimate of there being 300 million obese people in the world by 2025. In Brazil, it affects about 21% of the adult population and is mainly related to dyslipidemias [7].

In general, NCCDs are long-lasting, multiple, require permanent multidisciplinary follow-up, continuous interventions and demand the allocation of large material and human resources, which generates a heavy burden on the public health and social systems. Data from

the Ministry of Health reveal the Unified Health System (UHS) to spend R\$ 490 million every year for the treatments of diseases associated with obesity [8].

Metabolic Syndrome (MS), defined as a set of metabolic and hemodynamic abnormalities, frequently present in the obese individual, has also been highlighted in the literature in recent decades, in view of dyslipidemias as risk factors, with a worrying increase in the incidence of NCCD [9,10].

Studies have shown a significant decrease in markers of oxidative stress, weight reduction, lipid and glycemic profile, and the increase in HDL-c levels in camu-camu consumers, whereas these changes are not observed in synthetic vitamin C consumption [1].

Thus, the above mentioned Amazonian fruits are interesting sources of bioactive compounds and natural antioxidants, indicating promising prospects both for formulations of bioproducts and for a product to keep on retaining its bioactive substance's chemical stability, integrity and declared potency within the specified limits. The loss of chemical stability can be determined by intrinsic and extrinsic factors and lead to a change in the concentration of the active principle, leading to a decrease in the dose intended for consumption [11]. And in order to guarantee the estimated amount, as well as the stability of the antioxidant compounds of the above mentioned Amazonian fruits, the quality control of the manipulated capsules containing the nutritional supplement is of fundamental importance, so that the bioactive action keeps on being effective throughout the period it is being administered.

The present study has aimed to conduct the Amazonian fruits crude extracts-based encapsulation, keeping in mind their quality control, bioactive compounds physico-chemical characteristics and stability so as to warrant them to be used for the treatment of NCCD.

2. Material and Methods

2.1. Elaboration of the Supplement

The camu-camu (*Myrciaria dubia* (kunth) Mc Vaugh), açai (*Euterpe precatoria* Mart.) and guaraná (*Paullinia cupana*) fruits raw extracts were prepared at the Laboratory of Chemistry of Natural Products / UFAM and the laboratory of chemistry of natural products of the Amazonian Biotechnology Center (ABC) [12].

The process of elaboration of the supplement containing Amazonian fruits is under the protection of the National Institute of Industrial Property (NIIP) under the Process number: BR 10 2018 068302 0 (unpublished data).

2.2. Microbiological Analysis of Nutraceutical

Microbiological analyses were carried out in the Microbiology Laboratory (INPA), in the amount of 10 g of the nutraceutical, for quantification of total coliforms, fecal coliforms and fungi in triplicates, according to the recommendations of the American Public Health Association (APHA) [13], as well as the current legislation [14,15].

2.3. Centesimal Analysis

The determined formulation was analyzed in the Laboratory of the Coordination of Research in Food Technology - CPTA / INPA. The analyzes were performed in triplicate, and the results were expressed according to each method employed based on the principles of the Analytical Standards of the Adolfo Lutz Institute [16] and the Association of Official Analytical Chemistry [17].

The determination of the moisture in the formulation was carried out by direct heating in the oven at 105°C, followed by individual weighing's, in an analytical balance previously leveled and tared, until obtaining constant weight.

The determination of proteins were based on the determination of nitrogen through the Kjeldahl digestion process, which consists of three phases:

- Digestion - The organic matter in the sample is decomposed with sulfuric acid and a catalyst, where the nitrogen is transformed into ammoniacal salt. The digester used was the Tecnal TE-007 MP, 220 V.
- Distillation - Ammonia is released from the ammoniacal salt by reaction with hydroxide and received in an acid solution (saturated boric acid - 5 ml) of known volume and concentration.
- Titration - The amount of nitrogen present in the sample is determined by titrating the excess of the acid used in the distillation with hydroxide.

Since the nitrogen content of the different proteins is approximately 16%, the empirical factor 6.25 is introduced to convert the number of grams of nitrogen found in the number of grams of protein. And the value of the proteins was expressed in percentage, according to the equation (1) below [17]:

$$\frac{(V_a - V_b) \times 0.02 \times 0.014 \times f_v \times 6.25 \times 100}{P} = \text{protideos} \quad (1)$$

With:

V_a = sample volume,

V_b = white volume,

F_c = correction factor = 0.14,

P = g of the sample

Concentration of 0.02 M hydrochloric acid spent in titration

F_v = HCl conversion factor = 1.0526315789

The determination of lipids was carried out by extraction with hexane solvent, using a Soxhlet apparatus as extractor for 6 h [16].

The ashes of the samples were determined by incinerating the samples defatted and dried in a muffle at 550° C, with the aid of a EDGCON 1 P, 220V equipment, São Paulo, Brazil, for 4 h.

For crude fiber determination, the samples were desiccated and degreased, and the digestion procedures were used in acidic medium (1.25% H₂SO₄), followed by digestion in alkaline medium (1.25% NaOH) in the determiner of fiber TE-146 / 8-50 TECNAL, according to the method indicated by the Instituto Adolfo Lutz [16].

2.4. Estimated Energy Calculation

The carbohydrate content was estimated by the difference between 100 and the sum of the percentages of moisture, protein, total lipids, fibers and ashes.

The caloric value of the formulation was estimated from the centesimal composition using the following Atwater conversion factors: 9 kcal per gram of lipids, 4 kcal per gram of proteins, 4 kcal per gram of carbohydrates [18].

2.5. Stability of Phenolic Compounds, Ascorbic Acid (AA) and Antioxidant Activity of the Supplement

The analyses of the total phenolic compounds present in the formulation were analyzed by the Folin-Ciocalteu method with modifications [19]. The determination of AA was performed using the high performance liquid chromatography (HPLC) method [20].

In order to analyze the antioxidant activity, the DPPH sequestration method (IC₅₀), performing the microplate reading on the ultraviolet spectrometer at 517nm [21].

In addition to quantifying the supplement's active ingredients contents, monthly checks were carried out in the 6-month period.

2.6. Preparation of Capsules Containing the Supplement

The preparation of n. 00 capsules was performed according to the techniques used in the studies using 20% of microcrystalline cellulose to the mass of the supplement, as diluent and stabilizer of the bioactive substances of the supplement [22]. The filling of the capsules was performed by leveling them in an EMA 2000 brand semi-automatic encapsulating device, with capacity of 240 capsules per cycle, with four, 20 capsules batches being manipulated, so as to conduct the subsequent tests in triplicate. The d_{ap} was then calculated as the ratio of the mass of powder which filled the volume of 0.95 ml, which is the volumetric capacity of the n. 00 shell.

2.7. Analysis of the Quality Control of Hard n.00 Capsules Containing the Supplement

The capsules obtained by the master process were evaluated by the calculation of the mean weight (by non-destructive method), the relative standard deviation (or coefficient of variation), the percentage of variation of the theoretical content and also by the disintegration test following the description of the American Pharmacopoeia [23].

2.8. Average Weight

The Mean Weight (MW) was calculated as the arithmetic average of the individual weight of 20 units of capsules randomly selected within the manipulated batch. Then, the MW Standard Deviation (SD) was calculated to determine the relative standard deviation (RSD),

according to equation (2). For the analysis of the theoretical variation of the content of the capsules, among the 20 units that were individually weighed, the lowest and highest mass capsules were selected for the calculation of the Minimum Theoretical Quantity ($Q_{min. contend}$) and the Maximum Theoretical Quantity ($Q_{max. contend}$), respectively, through equations (3) and (4). Since the density relates mass and volume, the theoretical weight of the capsules (TW) was estimated by multiplying the apparent density (determined for the mixture of the camu-camu, açai and guaraná freeze-dried powder with 20% of microcrystalline cellulose), by the capacity of the chosen casing ($V_{caps.}$), Plus the mass of the empty enclosure, according to equation (5).

$$RSD = \frac{SD}{MW} \times 100 \quad (2)$$

$$Q_{min. contend} = \frac{m_{lightest caps.}}{TW} \times 100 \quad (3)$$

$$Q_{max. contend} = \frac{m_{heaviest caps.}}{TW} \times 100 \quad (4)$$

$$TW = (V_{caps.} \times d_{ap}) + m_{caps.} \quad (5)$$

2.9. Hard Capsule Disintegration Test

In the disintegration test, six units of each batch of capsules were used, adding each capsule in each basket, and subjected to the disintegration of Nova Ethics under the experimental conditions using a thermostat to maintain ultrapure water at 37°C, with constant frequency and specific path according to the instructions of the disintegrating apparatus.

2.10. Statistical Analysis

The experiment was conducted in a completely randomized design in triplicate. The study data was organized into a spreadsheet of Microsoft Excel 2010 © software.

The analysis of variance and the Tukey Honest Significance Difference (HSD) for multiple comparisons were performed in Prisma software, version 6. The level of significance used for all tests was 5% ($p < 0.05$).

3. Results and Discussion

3.1. Supplement's Microbiological Analysis

Microbiological analyses showed acceptable results within the limits established by Anvisa [14] for total coliforms, molds and yeasts, according to the recommendations of the American Public Health Association, APHA [13] and other current legislation [15]. The results obtained from the microbiological count (Table 1) showed that the sanitizing agent was effective in the elimination of pathogenic and deteriorating microorganisms, and that there was hygienic-sanitary safety in all processing stages for the raw stratum

preparation (raw material, prewash, sanitization, pulp, freezing and freeze-drying) and of the experimental ration (raw material, handling and time and temperature at the greenhouse).

Table 1. Microbiological counts on camu-camu, açai and guaraná sanitized with 5% sodium hypochlorite for 10'

| 5% sodium hypochlorite for 10' | | | |
|--------------------------------|-----------------|-----------------|--------|
| | Total coliforms | Fecal coliforms | Fungi |
| Temperature (°C) | 37 | 45 | 35 |
| Action Time | 24-48 h | 24-48 h | 5 days |
| MLN/g* | 0.0 | 0.0 | - |
| CFU/g** | - | - | <10 |

* MLN- Most Likelt Number

** CFU – Colony Forming Unit.

3.2. Centesimal Analysis of the Supplement

Table 2. Centesimal composition of the supplement, expressed g.100⁻¹

| Composition (%) | Values Average and SD |
|---------------------------|-----------------------|
| Moisture at 105° C | 15.44±1.13 |
| Proteins | 7.34±0.58 |
| Lipidis | 3.62±0.22 |
| Total Carbohydrate* | 67.83 |
| Ash | 3.65±0.98 |
| Fibers (g) | 2.12±0.45 |
| Total Energy Value (kcal) | 333.26 |

Values expressed in g.100⁻¹ of the nutraceutical, determinations in triplicate with their means and respective standard deviations.

* Calculation by difference.

The result obtained from the centesimal analysis of the nutraceutical is shown in Table 2. It is of fundamental importance to know the composition of the raw material in the application of different technological processes, as well as to interfere in the aspect of general quality,

influencing the sensorial attributes and the stability of the final product. The nutritional composition of the pericarp (pulp + peel) of the isolated camu-camu is considered to be of low content, on average with 0.82 ± 0.01 and $0.46 \pm 0.01\%$ [25,26].

According to Ordinance n. 27, dated January 13, 1998, from the Secretariat of Health Surveillance-SVS / MS, which recommends that for a solid food to have an adequate fiber source it must contain at least 3% of it in its composition. And in this study it was verified that the supplement presented 2.12 g fiber content.

3.3. Supplement's Bioactive Compounds Stability

The results showed there to be no correlation between the variables ($p > 0.05$) and the Smoothing Spline test for the stability of the bioactive compounds of the formulation for ascorbic acid (AA) and total phenols and for the activity antioxidant (DPPH) showed it to last for: 5, 4 and 6 months, respectively (Figure 1).

For a product to have chemical stability, each drug contained in it must maintain chemical integrity and stated potency within the specified limits. The loss of chemical stability can be determined by intrinsic and extrinsic factors and lead to a change in the concentration of the active principle, leading to a decrease on the dose destined to the patient. The generally accepted limit for the chemical decomposition of pharmaceutical products is no more than 10%, as long as the decomposition products are safely identified and their effects known in advance [11].

Studies on the stability of ascorbic acid in camu-camu found that its decomposition started at 60 days, with the time for the active concentration to decrease by 10% of the initial concentration under refrigeration temperature at 5 ° C, its microbiological quality [22].

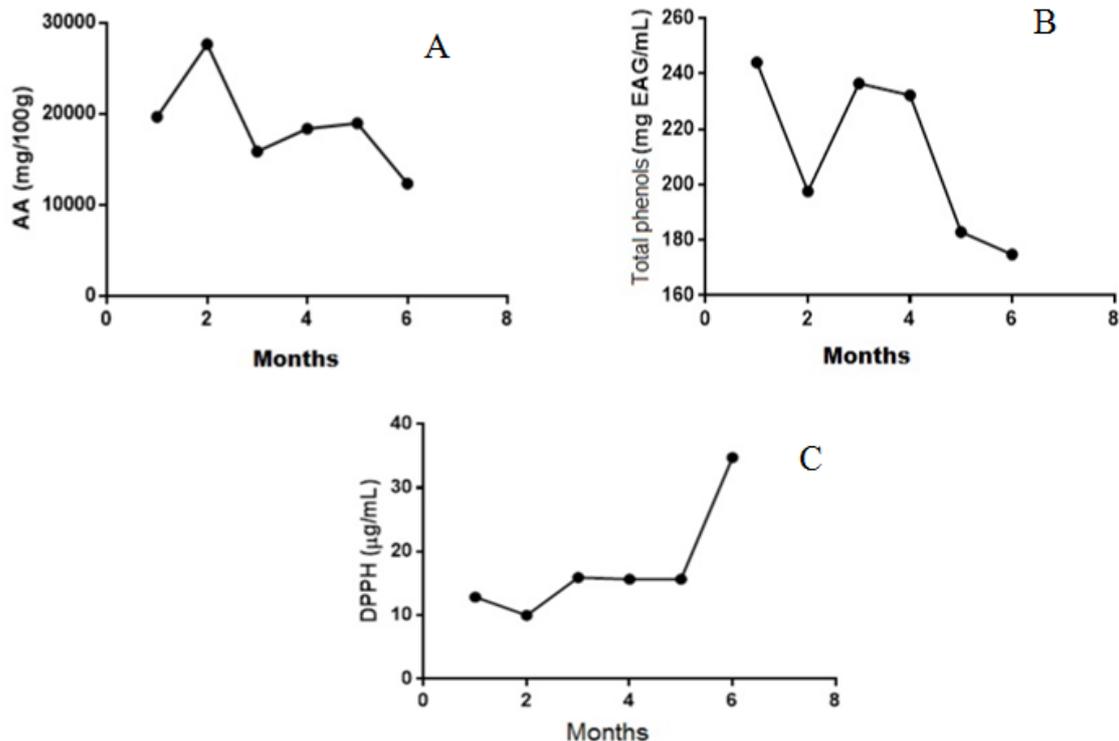


Figure 1. Stability of the bioactive compounds (A e B) and antioxidant activity of the supplement (C)

For a product to have chemical stability, each drug contained in it must maintain chemical integrity and stated potency within the specified limits. The loss of chemical stability can be determined by intrinsic and extrinsic factors and lead to a change in the concentration of the active principle, leading to a decrease on the dose destined to the patient. The generally accepted limit for the chemical decomposition of pharmaceutical products is no more than 10%, as long as the decomposition products are safely identified and their effects known in advance [11].

Studies on the stability of ascorbic acid in camu-camu found that its decomposition started at 60 days, with the time for the active concentration to decrease by 10% of the initial concentration under refrigeration temperature at 5 °C, its microbiological quality [22].

3.4. Quality Control Analysis of Hard Capsules

The calculated d_{ap} was 0.32 g / mL, i.e., a n. 00 capsule, which has a volumetric capacity of 0.95 ml, contains a mass of 0.2876 g of the powder resulting from the mixture of the lyophilized and crushed supplement of Amazonian fruits plus 20% (w / w) of microcrystalline cellulose. As the weight of the n. 00 capsule is 0.11213 g (determined by the mean of 20 units), the expected TW of each capsule (wrapper + mass) is 0.4089 g, and the active (active) supplement content found was 230 mg.

Table 3 shows the calculated values of MW , $RSDPR$, $Q_{min. content}$ and $Q_{max. content}$ for the 4 batches handled. Since filled capsules weigh less than 300 mg, batches where all individual capsule values are within the range of $\pm 10\%$ MW will be considered approved. Thus, since no unit had an individual mass value higher or lower than the limits calculated for each batch, all of them were considered approved in this enquiry. The lots were also considered approved with respect to the coefficient of variation or RSD , since the legislation recommends the calculated values not to exceed 4% [11].

Table 3. Calculated parameters for the quality control of the manipulated capsules

| Lot | MW (g) | SD | RSD (%) | Capsule lighter | | Capsule heavier | |
|-----|-------------|--------|--------------|-----------------|-----------------------|-----------------|-----------------------|
| | | | | m (g) | $Q_{teor.min}$ (%) | m (g) | $Q_{teor.max}$ (%) |
| 1 | 0.2877 | 0.0023 | 0.8000 | 0.3997 | 97.75 | 0.4122 | 100.81 |
| 2 | 0.2895 | 0.0035 | 1.2090 | 0.4068 | 99.49 | 0.4127 | 100.93 |
| 3 | 0.2868 | 0.0030 | 1.0460 | 0.4039 | 98.78 | 0.4100 | 100.27 |
| 4 | 0.2863 | 0.0041 | 1.4321 | 0.4045 | 98.92 | 0.4168 | 101.93 |

The $Q_{min. content}$ and $Q_{max. content}$ are calculated to allow estimation of acceptable capsule weight variation since, by the non-destructive method, the capsules were not opened to determine content homogeneity experimentally. Thus, assuming that the powder mass of the that has been encapsulated is perfectly homogeneous, the acceptable range of content must be contained in the range of 90 to 110%. As the minimum and maximum determined content showed to be 97.75% and 101.93%, respectively, all the lots were considered definitively approved for the study's follow up.

3.5. Hard Capsule Disintegration Test

The maximum time allowed for the hard capsules disintegration showed to be 45 minutes [24]. The results obtained in the disintegration of the samples are shown in Table 4. It is possible to observe that all the samples complied with the test specifications, with these being approved, the samples maximum and minimum disintegration time was 8 min and 50 sec and 6 min, respectively.

Table 4. Disintegration of master capsules

| n | Disintegration time |
|---|---------------------|
| 1 | 6 min and 00 sec |
| 2 | 7 min and 50 sec |
| 3 | 7 min and 10 sec |
| 4 | 8 min and 50 sec |
| 5 | 7 min and 20 sec |
| 6 | 7 min and 40 sec |

4. Conclusions

The stability analysis of the antioxidant compounds of the crude extracts of lyophilized and encapsulated Amazonian fruit followed the norms of the International Pharmacopoeia, obtaining its stability in the period of 5 months. In addition, it was found that the controlled stages of the encapsulation process and the conservation under refrigeration temperature at 5°C were important to guarantee the fruit's physical-chemical and microbiological quality.

It was also demonstrated in this study that the produced capsules containing the supplement were approved in the test of average weight and disintegration, guaranteeing the quality control and the efficiency of the bioproduct, since the antioxidant compounds are easily oxidized in the presence of oxygen, luminosity, environment temperature, extended storage time and packaging, among other factors.

Acknowledgments

The Authors thank the Federal University of Amazonas, the BIOPHAR, LIDETEF and LCQF laboratories, the National Research Institute of the Amazon for their support in physicochemical analyses and capsule quality tests, and the support provided by FAPEAM with the publication fee paid with PAPAC resource.

Funding

This work was not supported by any financial support.

Conflicts of Interest

The authors have no conflicts of interest to declare.

References

- [1] Langley, P.C., Pergolizzi, J.V., Taylor, R., Ridgway, C., "Antioxidant and Associated Capacities of Camu Camu

- (*Myrciaria dubia*): A Systematic Review”, *The Journal of Alternative and Complementary Medicine*, 21(1). 8-14. 2015.
- [2] Leão, A.L.M., Santos, L.C., “Consumo de micronutrientes e excesso de peso: existe relação?”, *Revista Brasileira de Epidemiologia*, 15(1). 85-95. 2012.
- [3] Yonekura, L., Martins, C.A., Sampaio, G.R., Monteiro, M.P., César, L.A.M., Miotto, B. M., Mori, C.S., Mendes, T.M.N., Ribeiro, M.L., Arçari, D.P., Torres, E.A.F.S., “Bioavailability of catechins from guaraná (*Paullinia cupana*) and its effect on antioxidant enzymes and other oxidative stress markers in healthy human subjects”, *Food & Function*, 7.2970-2978.2016.
- [4] Salomão-Oliveira, A., Lima, E.S.L., Marinho, H.A., Carvalho, R.P., “Benefits and Effectiveness of Using *Paullinia cupana*: A Review Article”, *Journal of Food and Nutrition Research*, 6(8). 497-503.2018.
- [5] Salomão-Oliveira, A., Costa, S.S., Marinho, H.A., “Metabolic syndrome: Intake of Camu-Camu (*Myrciaria dubia* (Kunth) McVaugh)”, *International Journal of Food Science and Nutrition*, 3(6). 5-13. 2018.
- [6] NCHS. National Center for Health Statistics. Health, United States, 2018. Hyattsville, MD. 2019.
- [7] Brasil. Ministério da Saúde. *Pesquisa Nacional de Saúde (PNS)*, Acesso e utilização dos serviços de saúde, acidentes e violências, grandes regiões e unidades da federação. IBGE, Coordenação de Trabalho e Rendimento, Brazil, Rio de Janeiro 100 p. 2015.
- [8] Brasil. Ministério da Saúde. Secretaria de Vigilância em Saúde. Departamento de Análise de Situação de Saúde, *Plano de ações estratégicas para o enfrentamento das doenças crônicas não transmissíveis (DCNT) no Brasil 2011-2022*, 2011.
- [9] NCEP-ATP III. Third Report of the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III), “Final Report, National Cholesterol Education Program, National Heart, Lung and Blood Institute, National Institute of Health”, *NIH Publication*, 2.5215.2002.
- [10] SBC. Sociedade Brasileira de Cardiologia, “Arquivos Brasileiros de Cardiologia”, *V Diretriz da Sociedade Brasileira de Cardiologia sobre Tratamento do Infarto Agudo do Miocárdio com Supradesnível do Segmento ST*, 105(2). 1-81.2015.
- [11] Brasil. Ministério da Saúde. Agência Nacional de Vigilância Sanitária, *Formulário Nacional da Farmacopeia Brasileira*, Brazil. 2. ed., 2012.
- [12] Rufino, M.S.M., Alves, R.E., Brito, E.S., Perez-Jimenez, J., Saura-Calixto, F., Mancini, F. J., “Bioactive compounds and antioxidante capacities of 18 non-tradicional tropical fruits from Brazil”, *Food Chemistry*, 121. 996-1002. 2010.
- [13] Sveum, W.H., Moberg, L.J., Rude, R.A., Frank, J.F., “Microbiological monitoring of the food processing environment”. In: Vanderzant C, Splittstoesser DF, Speck ML (Eds.), *Compendium of methods for the microbiological examination of foods*, 3. ed. Washington: APHA chapter, 3.51-74.1992.
- [14] ANVISA. Agência Nacional de Vigilância Sanitária, *Resolução RDC nº 12, de 02 de janeiro*, Brazil. 2001.
- [15] ICMS. Internation commission on microbiological specifications for foods, *Their significance and methods of enumeration*, 2. ed. University of Toronto. 2006.
- [16] IAL. Instituto Adolfo Lutz. *Normas Analíticas do Instituto Adolfo Lutz*, 4. ed., São Paulo. Brazil. 2008.
- [17] AOAC. Association of Official Analytical Chemistry, *Official methods of Analysis of the Association of Official Analytical Chemistry*, 18. ed., Gaithersburg, MD, International, 2005.
- [18] TBCA-USP. *Tabela de Composição Química dos Alimentos*. Universidade Federal de São Paulo. Brazil, versão 3. 2014.
- [19] Bonoli, M., Verardo, V., Marconi, E., Caboni, M. F., “Antioxidant phenols in barley (*Hordeum vulgare* L.) flour: comparative spectrophotometric study among extraction methods of free and bound phenolic compounds”, *Journal of Agricultural and Food Chemistry*, 52(16). 5195-5200. 2004.
- [20] Campos, F.M., Ribeiro, S.M.R., Della Lucia, C.M., Stringheta, P.C., Pinheiro-Sant’Ana, H.M., “Optimization of methodology to analyze ascorbic acid and dehydroascorbic acid in vegetables”, *Química Nova*, 32(1). 87-91.2009.
- [21] Sousa, C.M.M., Silva, H.R., Vieira-Jr, G.M., Ayres, M.C.C., Costa, C.L.S., Araújo, D.S.; Cavalcante, L. C. D.; Barros, E. D. S.; Araújo, P. B.M.; Brandão, M. S.; Chaves, M. H., “Total phenolics and antioxidant activity of five medicinal plants”, *Química Nova*, 30(2). 351-355. 2007.
- [22] Salomão-Oliveira, A., Costa, S.S., Dos Santos, K.S.C.R., Souza, T.P., Marinho, H.A., “Ascorbic acid from lyophilized camu-camu fruit: stability and quality control of hard capsules”, *Rev Ciênc Farm Básica Apl*, 37(1). 1-7. 2016.
- [23] The United States Pharmacopeia. *USP-NF 37 United States Pharmacopeia Convention*. 42. ed. 2019.
- [24] ANVISA. Agência Nacional de Vigilância Sanitária. *Farmacopeia Brasileira*. 5. ed. Brasília: Ministério da Saúde, 2. 524. 2010.
- [25] Yuyama, K.A., Valente, J.P. *Camu-camu*. 1. ed., PR: CRV, Curitiba, Brazil.2011.
- [26] Salomão-Oliveira, A., Marinho, H.A., “Bioactive Compounds and Antioxidant Activity of Camu-Camu (*Myrciaria dubia* (Kunth) Mc Vaugh) Grown on a Non-Flooded Land Ecosystem”, *Journal of Food and Nutrition Research*, 5(12). 941-946. 2017.
- [27] ANVISA. Agência Nacional de Vigilância Sanitária. *Resolução-RDC Nº 54, de 12 de novembro de 2012*. Dispõe sobre o Regulamento Técnico sobre Informação Nutricional Complementar. 2012.

