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Application of Brazil nut oil microparticles in a brownie recipe

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Abstract

The encapsulation of Brazil nut oil with rice protein and gum arabic as a wall material has been successfully demonstrated in previous studies carried out by this group. This process generated a product that maintains the functional properties of the oil and also can be used to produce other foods. In order to evaluate microparticles as a possible ingredient in food formulations, a brownie recipe was created containing encapsulated Brazil nut oil. The oil microparticles were produced by lyophilization and their physical characteristics were analyzed. They were added to the brownie recipe to assess the global acceptance and texture. Thermogravimetric analysis demonstrated the ability of the particles to withstand high temperatures (≅ 280 °C). The rheology tests demonstrated the Newtonian fluid behavior of the

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emulsion. The brownie containing the microparticles showed 8.05 ± 1.12 global acceptance and 7.71 ± 1.8 for texture, while the brownie with crude Brazil nut oil had 7.27 ± 1.76 global acceptance and 7.25 ± 1.85 for texture. These results make it possible to use the oil in new products, in addition to contributing to reduce the disposal of residues from the nut production process, as well as reducing the loss of quality of the oil.

Key words: *Bertholletia excelsa*, microencapsulation, sensory analysis, brownie, rice proteins.

1 INTRODUCTION

The Brazil nut (*Bertholletia excelsa*) is an oilseed consumed worldwide for its intrinsic nutritional qualities and gustatory appeal [1]. Products derived from Brazil nuts have been gaining space in the market, such as oil [2]. The crude oil in the seed consists of unsaturated (≅ 80%) and saturated (≅ 14%) fatty acids. The group of unsaturated fatty acids consists of around 25% monounsaturated fatty acids and 21% polyunsaturated fatty acids [1,3]. Several methods are used to conserve this oil to maintain the stability of its bioactive compounds.

Due to its good nutritional and commercial value, potentially able to provide benefits to human health, Brazil nut oil has become an important target of studies and investments. One of the ways to add value to Brazil nut oil is to apply technologies that increase its useful life and maintain and/or improve its natural characteristics. Encapsulation, for example, is a technique that has been applied in several areas, mainly in industrial sectors such as textiles, food production and pharmaceuticals. This process consists of storing compounds of interest by enclosing them in protective capsules, thus stabilizing the product during storage [4, 5]. The encapsulating material then becomes natural packaging, providing protection from damage caused by the environment and ensuring stability of the encapsulated product [6].

After being isolated from the natural state, vegetable oils, such as from Brazil nuts, are subject to lipid oxidation, which leads to loss of nutritional quality, in addition to the formation of unpleasant taste and the generation of toxic compounds harmful to health [7.8]. Therefore, the preservation of Brazil nut oil is necessary to maintain its original qualities.

Materials derived from natural products have been extensively investigated for medical applications as well as composition of new food products and cosmetics. The demand for protein concentrates has been growing due to their functionality and low price. These protein concentrates can be byproducts of the production of foods. The use of byproducts from food processing is a sustainable alternative for the use of residues, which would otherwise be discarded. Vegetable protein concentrates stand out due to the increased demand by consumers for sustainable technologies and the increase of restrictive diets for health reasons [9,10].

The success of using proteins as wall materials depends on their functional properties, not on their nutritional quality. The functional properties reflect how proteins will interact with other materials. These properties are related to the physical, chemical and conformational characteristics of proteins. Knowledge of the correlation between structure and functional properties of proteins is essential for their application in food making. Plant proteins are highly diverse and complex, thus attracting extensive research efforts [11].

A previous study demonstrated the good performance of rice proteins and gum arabic as a wall material for storing Brazil nut oil [12]. The protein fraction of rice grains is considered hypoallergenic and hypocholesterolemic [13]. The four most common protein fractions of rice are albumin, which has good solubility in water, globulin, which is soluble in saline solutions, glutelin, which has good solubility in acidic media, and prolamin, which is soluble in alcohol [14].

Therefore, microparticles consisting of Brazil nut oil, rice proteins and gum arabic (RCP+GA) can be used to preserve the functional compounds of the nut oil for inclusion in the formulation of different types of food products [12]. For this purpose, we evaluated the physical characteristics of the nut oil microparticles and used them in the formulation of a recipe for brownies, to assess their overall acceptance and texture compared to traditional brownies and those containing crude nut oil.

2 MATERIALS AND METHODS

2.1 Materials

The Brazil nut samples used in this work were collected according to the Brazilian biodiversity law under the National System for Genetic Heritage and Associated Traditional Knowledge Management (SisGen - AFAD928). The nuts were obtained from a processing plant in the state of Pará. The whole seeds, without shell and pelicule, were inserted in a mechanical press to obtain the oil. Then, the crude oil was left to stand, protected from light and refrigerated for 48 hours. The supernatant was collected and stored under refrigeration (-18 °C) in an amber flask until analysis. Rice protein concentrate (RCP) (GrowthSuplements, Brazil) and gum arabic (GA) (Nexira Brasil, Brazil) were used as wall materials.

2.2 Oil characterization

2.2.1 Parameters that determine the quality of the oil

The peroxide index and acidity index were determined to certify the oil quality [15]. The presence of aflatoxins was evaluated, for which purpose extraction was performed with methanol: water (55:45) and 0.1N hydrochloric acid. After stirring, centrifugation (15 minutes at 3000 g and 25 ºC) and filtration, a solution of sodium chloride (10%) and hexane was added to the sample. The sample was submitted to high-performance liquid chromatography (Column XBridge™ C18, 5 µm, 4.6 x 250 mm, flow of 0.8mL/min. with an injection volume of 40 µL), which allowed the detection of aflatoxins type B1, B2, G1 and G2 [15,16].

2.2.2 Characterization of fatty acids

In addition, the fatty acid profile was determined by gas chromatography according to the protocol of Hartman and Lago (1973) [17], with modifications. After extraction, the methyl esters resulting from the derivatization of the samples were analyzed in an Agilent 7890 chromatograph with a flame ionization detector operating at 280 ºC. The analysis was performed in an HP FFAP capillary column (25 m x 0.2 mm x 0.30 µm). The initial temperature was 150 ºC, which was increased to 180 °C at a rate of 30 °C/min, from 180 to 200 °C at

20 °C/min, from 200 to 230 °C at 3 °C/min, with the final temperature of 230 ° C held for 10 minutes. The pressure program was 15 psi for 10 min, increased to 25 psi at a rate of 5 psi/min and final pressure of 25 psi for 11 minutes. One µL of sample was injected into an injector heated to 250 °C operated in 1:50 flow division mode. Identification was performed by comparing retention times with the standards of NuChek Prep Inc. (Elysian, MN, USA) and quantification was performed by internal normalization.

2.3 Emulsion rheology

Rice protein concentrate, gum arabic and Brazil nut oil were homogenized in the proportions 2: 3: 1 in an Ultra-Turrax® homogenizer for 5 minutes at 10,000 rpm and 25 °C [12].The rheological behavior of the emulsion was evaluated using HAAKE MARS II oscillatory rheometer (Thermo Scientific, Karlsruhe, Germany) with titanium smooth plate-plate geometry with 60 mm diameter (PP60Ti), MARS II universal temperature controller (Thermo Scientific, Karlsruhe, Germany) at 25 ºC and gap of 1 mm. To obtain the flow curve, approximately 2 mL of emulsion was used in each analysis, with a shear rate that varied from 0 to 300 s^{-1}. The analyses were performed in triplicate and the data were adjusted to rheological models using the RheoWin Data Manager software, version 4750002 (Thermo Scientific, Karlsruhe, Germany).

2.4 Microencapsulation and characterization of particles

2.4.1 Microencapsulation

The emulsion was quickly frozen and subjected to freeze-drying (CHRIST model Alpha 1-2 LD plus, Germany). After lyophilization, the samples were ground manually into a fine powder (granulometry less than 100 mesh according to the US Standard). The microencapsulation yield of freeze-dried Brazil nut oil was calculated by the ratio between the total powder produced and the amount of dry material contained in the emulsion.

2.4.2 Thermogravimetric analysis

The thermal stability of Brazil nut oil particles with rice protein and gum arabic were evaluated in a TGA-200 automatic thermogravimetric analyzer (Las Navas Instruments, South Carolina, USA). The analyses were performed in a nitrogen atmosphere at a flow rate of 100 mL.min-1, with heating from 25 to 550 °C at a rate of 10 °C.min^{-1}. The analyses were performed in triplicate.

2.5 Sensory analysis and determination of the acceptability index

The sensory evaluation trials took place in the sensory analysis laboratory of Embrapa, respecting the recommendation of Brazil's National Health Council (CNS) Resolution 466/12, after the volunteers had signed the informed consent form (project approved by the relevant ethics committee under number CAAE 17721219.1.0000.9047. Before sensory analysis, the brownies were evaluated for the presence of microorganisms (coliforms at 45 °C and 35 °C, salmonella spp., filamentous fungi and yeasts, aerobic mesophiles and *Bacillus cereus*) [18].

A total of 108 untrained tasters were recruited who met the selection and inclusion criteria. The tasters were given three samples of brownies formulated with the following ingredients: wheat flour, eggs, brown sugar, cocoa powder, bar chocolate and chemical yeast. They differed by the presence of crude Brazil nut oil, encapsulated Brazil nut oil and traditional vegetable oil, used as a control. All were baked at an average temperature of 180 °C ± 20 for approximately 1 hour.

The tasters rated their acceptance of the product based on two attributes: overall acceptability and texture. The perception of these attributes was measured on a nine-point hedonic scale (1 = disliked extremely; 5 = neither liked nor disliked; 9 = liked extremely).

To calculate the acceptability index (AI), the results of the sensory analysis were applied in the expression: AI (%) = A x 100/B, where "A" refers to the average score obtained for the product attribute and "B" the maximum score given to the product attribute. For AI to be considered satisfactory, the attributes must have a result greater than or equal to 70% [19].

2.6 Statistical analysis

The data obtained in analyses were submitted to analysis of variance (ANOVA) and the Tukey test at 5%, using Excel® and R. The enabled evaluating the significant differences of the data.

3. RESULTS AND DISCUSSION

3.1 Oil characterization

Brazil nut oil showed 1.1 ± 0.13 mgKOH/g acidity and 1.3 ± 0.15 meq/Kg peroxide index after the extraction process. These values are well below what Brazilian regulations [20] recommend as a limit for acidity index (up to 4.0) and peroxides (up to 15.0) for unrefined pressed oils. The values of acidity and peroxides are reference parameters that attest to the quality and food safety of the oil [21]. Therefore, these values indicate the good state of conservation of the seeds and consequently of the oil [28]. In addition, they demonstrate that the oil extraction process did not cause oxidative damage to the byproduct. Values higher than those found in this study were reported by other authors, when characterizing nut oil extracted by ultrasound [22]. Extraction by hydraulic or mechanical pressing is the most common way to obtain Brazil nun oil. Besides being economical and practical, it preserves the original characteristics of the product [23,24].

Another way to check the quality of the oil is to evaluate the presence of mycotoxins, in particular aflatoxins, which are secondary metabolites of fungi, mainly from species Aspergillus species belonging to section Flavi, which can be found in nut seeds [27]. Depending on the dose, nutritional status, age and genus, these aflatoxins can lead to the appearance of a considerable number of pathologies. AFB_1, AFB_2, AFG_1 and AFG_2 are the most studied, and AFB_1 and AFG_1 are considered to have the greatest toxicogenic and carcinogenic power [25, 37]. The results indicated that the presence of aflatoxins of type AFG_1 and AFG_2 was not detected and traces of AFB_1 and AFB_2 were found below the detection limit (0.5 μg/Kg). Although current Brazilian regulations do not provide values for nut oil, the results found are low and demonstrated the quality of the product. Other authors have used the same extraction method to detect aflatoxins in decontaminated peanut oil samples, and noticed a reduction in contamination levels when applying the treatment [16]. This result confirms the effectiveness of the extraction method applied in this work. A study carried out with edible oils from Sudan, used the same extraction method as in the present study and detected different levels of aflatoxins [26].

The investigation of contaminants in Brazil nuts is common, since the extractive process can lead to contamination by microbiological agents that produce aflatoxins, thus generating contamination of the nut [27, 36]. The evaluation of the presence of aflatoxins in the nut oil carried out in this work confirmed its quality, since neither the nuts nor the oil underwent purification [39].

Gas chromatography allowed the evaluation of the profile of fatty acids present in the Brazil nut oil used in this study. **Table 1** shows the values for each fatty acid identified in this study, compared to studies carried out by other authors. It is possible to perceive the consistency of our results with the data presented in other studies. The results of the majority components are very similar to the reports in the literature. The presence and characteristics of these fatty acids vouch for the excellent quality of this byproduct. In addition, these results are in agreement with the peroxide and acidity index obtained, since there was no degradation of unsaturated fatty acids, indicated by comparison with other studies.

Table 1 - Fatty acids profile of Brazil nut oil

Fatty Acid		Oil used in this work	Santos et al. [23] *	Santos et al. [2]**	Colpo et al. [29]*
(C 12:0)	Lauric	-	-	0.767	-
(C 14:0)	Myristic	0.05 ± 0.00	0.06	0.565	0.07
(C 16:0)	Palmitic	14.15 ± 0.08	14.24	14.04	16.74
(C 16:1)	Palmitoleic	0.30 ± 0.00	0.01	0.016	0.43
(C 17:0)	Margaric	0.07 ± 0.00	-	-	0.14
(C 18:0)	Stearic	10.99 ±0.03	11.19	10.63	9.97
(C 18:1) (ϖ 9)	Oleic	37.6 ± 0.04	36.26	34.55	28.52
(C 18:2) (ϖ 6)	Linoleic	35.41 ±0.01	37.53	40.15	36.04
(C 18:3) (ϖ 3)	Linolenic	0.07 ± 0.00	0.08	0.085	0.11
(C 20:0)	Arachidonic	0.3 ± 0.00	-	-	0.17
(C 20:1) (ϖ 9)	Gadoleic	-	-	-	0.09
(C 22:0)	Behenic	-	-	-	-
C22: 1	Erucic	-	0.28	0.436	-
(C 22:2)	Docosahexaenoic	-	0.06	0.108	-

Data are expressed as mean ± SD (n = 3). * Oil extracted by organic solvent, ** Oil extracted by supercritical CO_2/(-) = Not supplied by the authors (ϖ 9) = omega 9, (ϖ 6) = omega 6, (ϖ 3) = omega 3/Profile displayed in percentage values.

3.2 Rheological Behavior

The rheological properties reveal the relationship between stress and deformation of a given material. This relationship can generate important information about the structure, stability and possible impacts on the manufacturing process of many products [30]. **Figure 1** shows the rheological behavior of the emulsion in this study at 25 ºC. The emulsion was classified as having Newtonian behavior (r^2 = 0.9995), according to the viscosity, which was constant (0.11 Pas ± 0.00) regardless of the shear rates. The small variation in viscosity may be associated with better stability in conditions of thermal stress. Therefore, regardless of the tension to which the fluid is subjected during a given processing type, the viscosity and resistance to flow will be the same, as long as the temperature and pressure conditions are maintained.

Newtonian behavior was also observed by other authors working with emulsions containing linseed oil, gum arabic and whey protein concentrate [31]. The literature also reports the Newtonian behavior of emulsions composed of avocado oil and maltodextrin/whey isolate as wall material [32]. Vegetable proteins have the same rheological behavior as whey proteins, making them alternatives to the use of animal proteins.

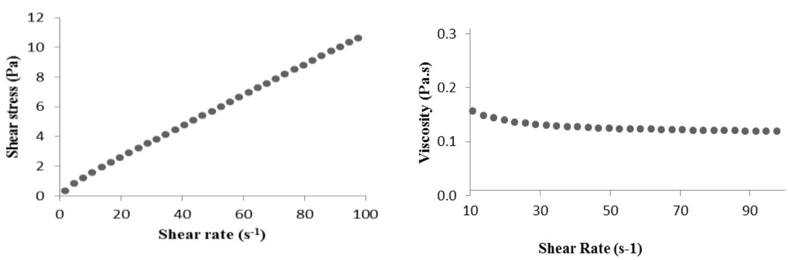

Figure 1: Rheological behavior and viscosity of emulsion with protein concentrate of rice, Brazil nut oil and gum arabic. Data are expressed as mean (n = 3).

3.3 Thermogravimetric analysis

Figure 2 shows the results obtained from the thermogravimetric analysis carried out on Brazil nut oil particles with rice protein concentrate. The particles showed greater loss of mass starting from 280 ºC. [33] performed thermogravimetric analyses of isolated soy protein and noticed a reduction in mass starting at 200 ºC. This same behavior was observed in another work with pure soy protein isolate, with a reduction in the range of 200 to 400 ºC [34]. The thermal stability of pure gum arabic was evaluated in another study, and the mass loss was observed in the range of 200 to 400 ºC [35].

In the present study, the particles showed greater thermal resistance than the other wall materials alone (compared with results obtained by the mentioned authors for another isolated plant protein).

We believe the compounds present in the microparticles were protected by the wall material, since encapsulation increases the thermal resistance of the particles [35].

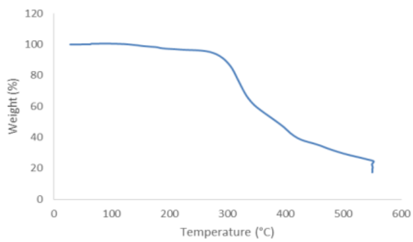

Figure 2: Thermogravimetric behavior of microparticles of Brazil nut oil, RCP + GA. Data is expressed as mean (n = 3).

3.4 Sensory analysis

The microbiological safety of the three brownie recipes analyzed was confirmed the tests, which that found the absence of *Salmonella* spp. and *Bacillus cereus*, and non-significant quantities of aerobic mesophilic bacteria (<5.0 x 10 CFU/mL), total fungi (<1.0 x 10 CFU/mL) and coliforms at 35 ºC and 45 ºC (<1.0 x 10 CFU/mL).

The participants of the taste trial were 65.74% women and 34.26% men. The majority of them were graduate students (61.11%), with ages between 26 and 45 years (56.48%). The evaluators reported the frequency of individual consumption of brownies, and 54.63% reported consuming them rarely and 44.44% reported consuming them frequently. Even though they were untrained evaluators, most were able to identify important differences between the products. This behavior was assessed according to the individual free comments about each sample reported by the tasters. **Figure 3** shows the distribution of comments about what consumers liked and disliked, gathered in groups of attributes for each sample.

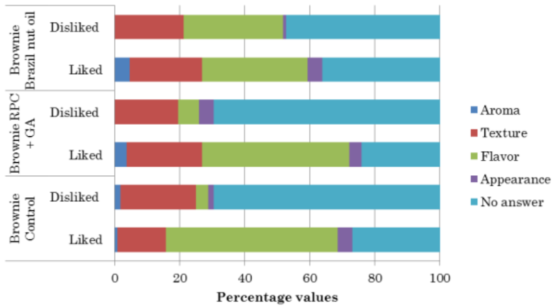

Figure 3: Frequency of the comments about the attributes liked and disliked in the brownies evaluated by consumers.

Table 2 shows the average favorable scores of the brownies: control, with microparticles of Brazil nut oil + RCP+GA and with crude Brazil nut oil only. The statistical treatment of the data revealed there were no significant differences in overall acceptability (p-value <0.05) between the control brownie and the one containing the microparticles. The brownie containing crude nut oil was less accepted by the evaluators (p-value <0.05). The acceptability index corroborates these values. In general, these results reflect the good acceptance of the new product compared to the traditional recipe.

Table 2: Results of acceptance evaluation of brownies with microparticles of Brazil nut oil + RCP+GA.

Samples	Overall acceptability	Texture	Acceptability Index (%)
Control brownie	*8.16 ± 1.15*^{a}	*7.23 ± 1.5*^{a}	*90.7*
RPC+GA brownie	*8.05 ± 1.12*^{a}	*7.71 ± 1.8*^{a}	*89.4*
Brazil nut oil brownie	*7.27 ± 1.76*^{b}	*7.25 ± 1.85*^{a}	*80.8*

a, b, c, d different letters represent significant differences (p-value<0.05). Results are presented as mean ± standard deviation (n=3). RCP+GA: rice protein concentrate + gum arabic.

These results also reveal that the change in the brownie formulation with the addition of RCP + GA microparticles did not negatively influence the product. Most tasters reported consuming brownies (rarely or frequently), which means that a large number of those in this study knew how to identify significant differences in the product. In addition, it can be seen that the direct addition of Brazil nut oil in

brownies may have negatively affected the product (which showed less acceptability). Brazil nut oil is a product with great nutritional and functional value, due to its composition, especially unsaturated fatty acids [2, 29]. However, the exposure of these fatty acids to heat, moisture, oxygen and light present in the environment can lead to loss of product quality and important sensory changes [12]. The RCP + GA microparticles probably promoted the protection of the Brazil nut oil's components against such negative effects of the production process, likely reflected in the better acceptance of the product. The brownie containing the RCP + GA microparticles is an alternative product that met consumer expectations. This product has components of Brazil nut oil in proper for consumption, which can generate health benefits.
The values shown in **Table 2** demonstrated the maintenance of the oil inside the particles during the cooking process, which possibly led to a gradual release of the oil into the mixture. The thermogravimetric analysis (section 3.3) of RPC + GA revealed that the particles started losing weight at approximately 270 ºC. The brownies were baked at 180º C, so the particles still maintained their structures and prevented possible oxidative damage to the oil. After tasting the brownies, the participants stated in few words what attributes they most liked and disliked in the brownies. These answers were gathered in five categories: aroma, texture, flavor, appearance and no answer, as indicated in **Figure 3.** The flavor of the brownie with Brazil nut crude oil was disliked in higher proportion (% of comments), which can be related to the thermal stress suffered by the oil during baking.

4 CONCLUSIONS

Studies to improve the performance of use of plant proteins as wall materials are still needed. However, the results of this study showed that encapsulation of Brazil nut oil in particles made of vegetable protein and gum arabic helped to maintain the properties of the oil. The reuse of byproducts, such as Brazil nut oil, reduces the production of wastes and generates an alternative product for consumption of functional components.

The RPC + GA particles used to encapsulate the Brazil nut oil employed in the brownie recipe generated a high-quality product with the same characteristics as the product traditionally consumed, according to the evaluators. This reiterates that the Brazil nut oil encapsulated in RCPA+GA particles analyzed in this study can be used as an ingredient in food formulations and/or supplements in specific diets.

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Declaration of conflict of interest

The authors declare there are no conflicts of interest.

REFERENCES

[1] Venkatachalam, M., Sathe, S. K., 2006. Chemical composition of selected edible nut seeds. Journal of agricultural and food chemistry, 54(13), 4705-4714. https://doi.org/10.1021/jf0606959.

[2] Santos, O. V. D., Corrêa, N. C. F., Carvalho Jr, R. N., Costa, C. E. F., Lannes, S. C. D. S., 2013. Yield, nutritional quality, and thermal-oxidative stability of Brazil nut oil (Bertolletia excelsa HBK) obtained by supercritical extraction. Journal of food engineering, 117(4), 499-504. https://doi.org/10.1016/j.jfoodeng.2013.01.013.

[3] Stockler-Pinto, M. B., Carrero, J. J., Weide, L. D. C. C., Cozzolino, S. M. F., Mafra, D.,2015. Effect of selenium supplementation via Brazil nut (Bertholletia excelsa, HBK) on thyroid hormones levels in hemodialysis patients: a pilot study. Nutricion hospitalaria, *32*(4), 1808-1812. htpp://10.3305/nh.2015.32.4.9384.

[4] Martins, I. M., Barreiro, M. F., Coelho, M., Rodrigues, A. E., 2014. Microencapsulation of essential oils with biodegradable polymeric carriers for cosmetic applications. Chemical Engineering Journal, 245, 191-200. https://doi.org/10.1016/j.cej.2014.02.024.

[5] Paulo, F., Santos, L., 2017. Design of experiments for microencapsulation applications: A review. Materials Science and Engineering: C, 77, 1327-1340. https://doi.org/10.1016/j.msec.2017.03.219

[6] Nazzaro, F., Orlando, P., Fratianni, F., Coppola, R., 2012. Microencapsulation in food science and biotechnology. Current opinion in biotechnology, 23(2), 182-186. https://doi.org/10.1016/j.copbio.2011.10.001.

[7] Kerrihard, A. L., Nagy, K., Craft, B. D., Beggio, M., Pegg, R. B., 2015. Oxidative stability of commodity fats and oils: Modeling based on fatty acid composition. Journal of the American Oil Chemists' Society, *92*(8), 1153-1163. https://doi.org/10.1007/s11746-015-2686-4.

[8] Li, J., Liu, J., Sun, X., Liu, Y., 2018. The mathematical prediction model for the oxidative stability of vegetable oils by the main fatty acids composition and thermogravimetric analysis. *LWT*, *96*, 51-57. https://doi.org/10.1016/j.lwt.2018.05.003.

[9] Malik, M. A., & Saini, C. S., 2018. Rheological and structural properties of protein isolates extracted from dephenolized sunflower meal: Effect of high intensity ultrasound. Food Hydrocolloids, 81, 229-241. https://doi.org/10.1016/j.foodhyd.2018.02.052.

[10] Tansaz, S., Schulte, M., Kneser, U., Mohn, D., Stark, W., Roether, J. A. Cicha, I., Boccaccini, A. R. (2018). Soy protein isolate/bioactive glass composite membranes: Processing and properties. European Polymer Journal, *106*, 232-241. https://doi.org/10.1016/j.eurpolymj.2018.07.003.

[11] Altschul, A. M., & Wilcke, H. L. (Eds.), 2013. New Protein Foods: Seed Storage Proteins (Vol. 5). Academic press.

[12] Oliveira, T. S. Microencapsulação e estabilidade oxidativa do óleo de castanha-do-Brasil (Bertholletia excelsa) com concentrados proteicos vegetais. (Dissertação Mestrado em Ciências Farmacêuticas) Universidade Federal de Amazonas. Amazonas, 2019.

[13] Ju, Z. Y., Hettiarachchy, N. S., Rath, N., 2001. Extraction, denaturation and hydrophobic properties of rice flour proteins. Journal of food science, 66(2), 229-232. https://doi.org/10.1111/j.1365-2621.2001.tb11322.x.

[14] Shih, F. F., 2003. An update on the processing of high-protein rice products. Food/Nahrung, *47*(6), 420-424.https://doi.org/10.1002/food.200390093.

[15] AOAC, 2016. Official methods of analysis. In A. of O. A. Chemists (Ed.). (19th ed.). Maryland: AOAC.

[16 Magzoub, R. A. M., Yassin, A. A. A., Abdel-Rahim, A. M., Gubartallah, E. A., Miskam, M., Saad, B., Sabar, S., 2019. Photocatalytic detoxification of aflatoxins in Sudanese peanut oil using immobilized titanium dioxide. *Food Control*, *95*, 206-214. https://doi.org/10.1016/j.foodcont.2018.08.009.

[17] Hartman, L., Lago, R. C., 1973. Rapid preparation of fatty acid methyl esters from lipids. Laboratory practice, 22(6), 475-6. https://doi.org/10.1016/j.foodhyd.2015.01.001.

[18] APHA – American Public Health Association, 2001. Compendium of methods for the microbiological examination of foods. (4th ed.). Washington, (Chapter 7).

[19] Teixeira, L. V., 2009. Análise sensorial na indústria de alimentos. Revista do Instituto de Laticínios Cândido Tostes, 64(366), 12-21.

[20] BRASIL. Agência Nacional de Vigilância Sanitária. Resolução-RDC n. 7, de 18 de fevereiro de 2011. Dispõe sobre limites máximos tolerados para micotoxinas em alimentos. Diário Oficial de Brasília. Seção 1, p. 66-67, 2011.

[21] Santos, A. L., Kunrath, N. F., Souza, I. S., Carvalho, C. E. G., 2015. Avaliação físico-química do óleo residual da castanha-do-brasil (Bertholletia excelsa HBK) e sua conversão em biodiesel. Rev. Encicl. Bras, 11(22), 3550-3561. http://dx.doi.org/10.18677/Enciclopedia_Biosfera_2015_064.

[22] Schons, J. I., Fiori, K. P., Ribeiro, E. B., Andrighetti, C. R., Nogueira, R.,de Sousa Valladão, D. M., 2017 . Extração assistida por ultrassom e caracterização do óleo da castanha-do-brasil (Bertholletia excelsa HBK). Interciencia, 42(9), 586-590.

[23] Santos, O. V. D., Corrêa, N. C. F., Soares, F. A. S. D. M., Gioielli, L. A., Costa, C. E. F., Lannes, S. C. S., 2012. Chemical evaluation and thermal behavior of Brazil nut oil

obtained by different extraction processes. Food Research International, 47(2), 253-258. https://doi.org/10.1016/j.foodres.2011.06.038.

[24] Rodrigues, J. E., Araújo, M. E., Azevedo, F. F. M., Machado, N. T., 2005. Phase equilibrium measurements of Brazil nut (Bertholletia excelsa) oil in supercritical carbon dioxide. The Journal of supercritical fluids, 34(2), 223-229. https://doi.org/10.1016/j.supflu.2004.11.018.

[25] Hedayati, M. T., Pasqualotto, A. C., Warn, P. A., Bowyer, P., Denning, D. W., 2007. Aspergillus flavus: human pathogen, allergen and mycotoxin producer. Microbiology, 153(6), 1677-1692. https://doi.org/10.1099/mic.0.2007/007641-0.

[26 Idris, Y. M., Mariod, A. A., Elnour, I. A., Mohamed, A. A., 2010. Determination of aflatoxin levels in Sudanese edible oils. Food and Chemical Toxicology, 48(8-9), 2539-2541. https://doi.org/10.1016/j.fct.2010.05.021.

[27] Álvares, V. D. S., Castro, I. M. D., Costa, D. A. D., Lima, A. C. D., Madruga, A. L. S., 2012. Qualidade da castanha-do-brasil do comércio de Rio Branco, Acre. Acta Amazônica, 42(2), 269-274. https://doi.org/10.1590/S0044-59672012000200013.

[28] Fiori, K. P., Torres, M. D. P. R., Schons, J. I., Ribeiro, E. B., Nogueira, R. M., Vasconcelos, L. G., Valladão, D. M. D. S., 2017. Microemulsion of Brazil nut oil as a natural product to improve superoxide release in human phagocytes. Química Nova, 40(9), 1051-1057. https://doi.org/10.21577/0100-4042.20170113.

[29] Colpo, E., Vilanova, C. D. D., Reetz, L. G. B., Duarte, M. M., Farias, I. L. G., Meinerz, D. F., Wagner, R., 2014. Brazilian nut consumption by healthy volunteers improves inflammatory parameters. Nutrition, 30(4), 459-465. https://doi.org/10.1016/j.nut.2013.10.005.

[30] Secolin, V. A., Souza, C. R., Oliveira, W. P., 2017. Spray drying of lipid-based systems loaded with Camellia sinensis polyphenols. Journal of liposome research, 27(1), 11-20. https://doi.org/10.3109/08982104.2016.1140183.

[31] Tonon, R. V., Pedro, R. B., Grosso, C. R., Hubinger, M. D., 2012. Microencapsulation of flaxseed oil by spray drying: Effect of oil load and type of wall material. Drying Technology, *30*(13), 1491-1501. https://doi.org/10.1080/07373937.2012.696227.

[32] Bae, E. K., Lee, S. J., 2008. Microencapsulation of avocado oil by spray drying using whey protein and maltodextrin. Journal of Microencapsulation, 25(8), 549-560. https://doi.org/10.1080/02652040802075682.

[33] Nesterenko, A., Alric, I., Silvestre, F., Durrieu, V., 2012. Influence of soy protein's structural modifications on their microencapsulation properties: α-Tocopherol microparticle preparation. *Food Research International*, *48*(2), 387-396. https://doi.org/10.1016/j.foodres.2012.04.023.

[34] Guerrero, P., Retegi, A., Gabilondo, N., De la Caba, K., 2010. Mechanical and thermal properties of soy protein films processed by casting and compression. Journal of Food Engineering, 100(1), 145-151. https://doi.org/10.1016/j.jfoodeng.2010.03.039.

[35] Westphalen, G., de Freitas, V. O., Rosa, V. R. E. G., 2015. Encapsulação dos compostos bioativos do Butia quaraimana a partir da técnica de liofilização. Blucher Chemical Engineering Proceedings, 1(3), 1986-1991.

[36] Ribeiro, M. S.S., Silva, S.H.M., Freitas-Silva, O., Abreu, L.F. Lima, C.L.S. 2019. Identification and characterization of aflatoxigenic Aspergillus Section Flavi by polyphase approach from Brazil nuts produced in agroforesty production

system. Emirates Journal of Food and Agriculture. 31, 10 (Oct. 2019), 768-772. https://doi.org/https://doi.org/10.9755/ejfa.2019.v31.i10.2017.

[37] Freitas-Silva, O. ,Venâncio, A. 2011. Brazil nuts: benefits and risks associated with contamination by fungi and mycotoxins. Food Research International, 44(5), 1434-1440. https://doi.org/10.1016/j.foodres.2011.02.047.

[38] Ribeiro, M. S. S., Freitas-Silva, O., Castro, I. M., Teixeira, A., Marques-da-Silva, S. H., Sales-Moraes, A. C. S. Abreu, L.F., Sousa, C. L. 2020. Efficacy of sodium hypochlorite and peracetic acid against Aspergillus nomius in Brazil nuts. Food Microbiology, *90*, 103449. https://doi.org/10.1016/j.fm.2020.103449.