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Antioxidant capacity of guaraná (Paullinia cupana) microcapsules

VINICIUS CAVALCANTI
https://orcid.org/0000-0003-1843-0757
Federal University of Amazonas
Biodiversity and Biotechnology Network of the Legal Amazon
Human Performance Laboratory (LEDEHU)
JOSIANA MOREIRA MAR
https://orcid.org/0000-0003-4442-6874
Laboratory of Nanostructured Polymers (NANOPOL)
EDGAR APARECIDO SANCHES
https://orcid.org/0000-0002-1446-723X
Federal University of Amazonas
Laboratory of Nanostructured Polymers (NANOPOL)
WHENDEL MESQUITA DO NASCIMENTO
https://orcid.org/0000-0001-7556-4423
Biodiversity and Biotechnology Network of the Legal Amazon
AGNELO WEBER DE OLIVEIRA ROCHA
https://orcid.org/0000-0003-2161-9470
Biodiversity and Biotechnology Network of the Legal Amazon
IVAN DE JESUS FERREIRA
https://orcid.org/0000-0001-7895-5905
Federal University of Amazonas
[2]Biodiversity and Biotechnology Network of the Legal Amazon
DAURIMAR PINHEIRO LEÃO
https://orcid.org/0000-0002-9242-8922
Federal University of Amazonas.
Biodiversity and Biotechnology Network of the Legal Amazon
PEDRO HENRIQUE CAMPELO FÉLIX
https://orcid.org/0000-0002-5137-0162
Federal University of Amazonas
CINTIA MARA COSTA DE OLIVEIRA
https://orcid.org/0000-0002-1065-8418
Program in Biodiversity and Biotechnology Network of the Legal Amazon
Multidisciplinary Program in Biotechnology

Abstract

The powdered guarana fruit (Paullinia cupana) has a high content of catechins, polyphenols with antioxidant action that are easily

absorbed by the body. According to the evidence of this potential and the beneficial effects they have, microencapsulation by ionic gelation becomes a potential and promising market. In this context, this study aims to evaluate the antioxidant capacity of the microencapsulated guarana (Paullinia cupana), verifying the efficiency of carrier agents, considering the preservation of physical and chemical properties. The samples and carrier agents used were for the treatment T1 (guarana + sodium alginate), T2 (guarana + sodium alginate + inulin), T3 (guarana + sodium alginate + whey protein isolate) and T4 (guarana + sodium alginate + maltodextrin). The results were analyzed using statistical data and it was observed that there was a superior encapsulation of the T4 compound compared to the other microcapsules.

Keywords: Methylxanthines; Tannins; Microencapsulated.

1. INTRODUCTION

Technological advances in the food industry have caused an increase in concerns about food security. Researchers have applied nanotechnology to develop from smart packaging, product tracking and, more recently, bioactive microencapsulations.

Guarana (*Paulinia cupana*), a native fruit of the Amazon region whose seeds have always been used as a stimulant and as a traditional remedy by indigenous tribes in Brazil over the years, has come to be consumed as soft drinks, energy drinks and supplements (Mendes & Carlini, 2007; Smith & Atroch, 2010a). In this trend, the stimulating, tonic and aphrodisiac factors found in guarana are therapeutic properties that became known worldwide from the first recorded indigenous reports, which led to the inclusion of guarana in the list of medicinal plants, in addition to the increased use and commercialization (Schimpl et al., 2013).

In the health area, scientific investigations have shown that the consumption of guarana has possible positive effects related to cardiovascular metabolic diseases, linked to lipid metabolism and low density lipoprotein oxidation (Portella et al., 2013), antioxidant biological activity of the polysaccharides (Dalonso & Petkowicz, 2012),

protective effects on NIH-3T3 fibroblasts (Bittencourt et al., 2013) and improvement of breast cancer patients who have undergone chemotherapy treatments (Oliveira Campos et al., 2011).

On the other hand, the practice of incorporating food into bioactive systems has been a routine in the food industry. Among the most innovative techniques for this process is the ion gelling technique that is used for microencapsulation of sensitive compounds, given its simplicity and versatility (Benavides et al., 2016).

Ionic gelling is a physical-chemical process based on ionic interactions between compounds of opposite charges (Saravanan & Rao, 2010). The production of microspheres without the use of organic solvents has become increasingly promising, especially for microencapsulation of drugs and thermosensitive active compounds of interest to the food industry (Patil, 2010).

Therefore, based on the above, this study aims to evaluate the antioxidant capacity of the microencapsulated guarana (Paullinia cupana), verifying the efficiency of carrier agents, considering the preservation of physical and chemical properties.

2. MATERIAL E METHODS

2.1. Characterization of guarana powder using the HPLC technique

To carry out this study, guarana powder, harvest 2017/2018 was used, acquired through producers in the county of Maués (Latitude: 3 ° 22 '54' 'South, Longitude: 57 ° 42' 55 " West) , 259 km away from Manaus, capital of the State of Amazonas, whose location has one of the most traditional cultures referring to guarana in Brazil.

Simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) were prepared as described by Minekus et al. (2014). The reagents used were of analytical grade and purchased from Sigma-Aldrich (Germany).

The High Performance Liquid Chromatography (HPLC) technique was performed with a Shimadzu liquid chromatography; model: LC-6AD. Sigma Aldrich standards with specifications for theobromine, catechin, theophylline, caffeine and epicatechin were used. The separation of the guarana standards was performed with a

C18 (Varian) column, 250 x 4.6 mm, 5 µm, wavelength: 280 nm, injection volume: 20 µL, flow rate of the mobile phase: 1 mL / min, mobile phase: water: acetonitrile: methanol: ethyl acetate: acetic acid (89: 6: 1: 3: 1), pH 3.5.

A gradient system with two mobile phases with the following components was used: eluent A (water + 0.1% formic acid) and eluent B (methanol). The flow system was completed with 1.0 ml / min of raw extracts with a final injection volume of 60µL. The system operates at a temperature of 27ºC. The applied elution conditions included: 10% isocratic 0 to 4 min; Linear gradient between 10% to 50% of eluent B with injection time between 4 to 15 minutes; 50% isocratic B for 15-30 min and; finally, washing and conditioning the column. The absorbance measurements were read at 270 nm, 324 nm and 373 nm, respectively. The experiments were carried out in triplicates. Data collection was obtained at wavelengths of 270 nm and 280 nm.

2.2. Microencapsulation by ionic gelation

A portion of the guarana powder was thawed and vacuum filtered on Qualy® filter paper (J Prolab, São José dos Pinhais, Brazil), using a Büchner funnel. This procedure was carried out with the purpose of eliminating suspended solids (facilitating the passage through the atomizer nozzle) and reducing the lipid content, thus reducing the risks of oxidation of the product. Subsequently, the filtered guarana juice was divided into four aliquots, each representing a particulate system. To these systems the carrier agents were respectively added, in a concentration of 6%, until complete dissolution.

Guarana juice was obtained in a Turex food processor. Before using guaraná juice in the microencapsulation process, it was suspended in a sonicator (Branson, Danbury, United States), for 2 minutes at 200 W of power for homogenization

The analyses were performed in a half-light environment and with covered vials. To determine the content of methylxanthines and tannins in the guaraná microcapsules obtained, 0.3 g of the sample was diluted in 3.0 ml of cooled phosphate buffer in order to facilitate the release of the methylxanthines and tannins from the guarana and microcapsule structures.

The solution was transferred to a centrifuge tube covered with aluminum foil with 3.0 mL of acetone: ethyl alcohol solution (1: 1). Followed by centrifugation at 1850 x g for 5 minutes. Four washes were performed with the same acetone solution: ethyl alcohol, the supernatant being transferred to a flask with the addition of 10 ml of distilled water and 5.0 ml of petroleum ether. The supernatant was transferred to a covered volumetric flask and the volume made up with petroleum ether.

The sodium alginate was dissolved in the guarana juice. After total dissolution, the whey protein isolate, or maltodextrin or inulin was added. The guarana solutions with the wall materials were submitted to an ultrasonic bath (Unique, model US 2800, São Paulo, Brazil) for 20 minutes to remove occluded air. Afterwards, the solutions were atomized in spray equipment (model MSD 1.0; Labmaq do Brasil, Rio Preto, Brazil) with a double fluid pressurized nozzle, fed by a peristaltic pump at a flow rate of 0.7 L / h, determined by pre- tests previously performed at an air flow of 30 L.min-1, as recommended by the manufacturer. The droplets formed, as soon as atomized, came into contact with a solution of calcium chloride, with a concentration of 1.0 g / 100 mL, at a distance of 15 centimeters. The particles were kept in this solution for 20 minutes, in order to guarantee the formation of the hydrogel structure, followed by decantation and washing of the microcapsules with distilled water.

The carrier agents used were: maltodextrin 30DE, inulin and protein isolated from whey. Four formats of particulate systems were prepared, according to Table 01, being: 1 (Guaraná + sodium alginate), 2 (guaraná + sodium alginate + inulin), 3 (guaraná + sodium alginate + whey protein isolated) and 4 (guarana + sodium alginate + maltodextrin)

Table 01. Composition of guaraná and sodium alginate solutions to obtain microcapsules via ionic gelling. Source: Authors themselves.

Treatment	**Wall material (g.100mL of solution)**			
	Sodium alginate	Alg + Inulin	Alg + Isolated	Alg + Maltodextrin
1	1,5 g	-	-	-
2	-	1,5 g + 1,5 g	-	-
3			1,5 g + 1,5 g	-
4	-	-	-	1,5 g + 1,5 g

2.3. Characterization of the microcapsule by scanning electron microscopy (SEM)

To obtain the microstructural morphological characterization and identification of chemical elements of the guarana particulate system, the microcapsules were observed in a scanning electron microscope TM 3000 Tablet opmicroscope (Hitachi, Tokyo, Japan), from the Electron Microscopy Laboratory of the National Research Institute of the Amazon (INPA). The samples were accommodated in double-sided carbon tapes (TedPella, Inc., Redding, USA), and then fixed in an aluminum sample holder (stub) and dried at room temperature for 24 hours. Then, an electronic scan analysis was performed to obtain the three-dimensional image of the samples.

2.4. Determination of antioxidant activity

Radical Elimination Test (DPPH)

The ability of the extracts to donate an electron and eliminate the 2,2-diphenyl-1-picryl-hydrazil radical (DPPH) was determined by the method of Brand-Williams et al. (1995). The DPPH radical scavenging activity was presented as a function of the Trolox - Trolox concentration equivalent antioxidant capacity (TEAC), being defined as Trolox concentration with equivalent antioxidant activity expressed as µM per mL (µM TE / mL).

Radical Elimination Test (ABTS)

The radical elimination activity of the extracts against 2,2´-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS • +) was estimated according to Re et al., (1999). Briefly, the ABTS radical cation (ABTS • +) was produced by reacting the ABTS stock solution (7 mM) with 2.45 mM potassium persulfate concentration) and allowing the mixture to remain in the dark at room temperature for 12 to 16 hours before use. The ABTS • + solution was then diluted with ethanol to an absorbance of 0.7 ± 0.02 at 734 nm and equilibrated at 30 ° C. After adding 1.0 mL of ABTS • + solution diluted to 0, 01 mL of samples, the absorbance reading was performed at 30 ° C after 6 min. The results were expressed as the TEAC value (µM TE / mL).

Ferric antioxidant power test (FRAP)

The ferric antioxidant power test (FRAP) was carried out according to the procedure of Benzie & Strain (1999) with slight modification. The absorbance was recorded at 593 nm and the results were expressed as µM TE / mL.

2.5. Statistical analysis

All tests were performed in triplicate and the parametric ANOVA test and the Tukey test were used.

3. RESULTS

3.1. Chemical characterization of guarana powder

The average of the results regarding the characterization and concentrations of methylxanthines and tannins obtained by the HPLC-HPLC analysis are shown in Figure 01 and Table 02.

The chromatogram was obtained from five standard solutions, theobromine, catechin, theophylline, caffeine and epicatechin. Each solution obtained the following retention time: theobromine (10.1 min), catechin (13, 7 min), theophylline (14.1 min), caffeine (17.1 min), and epicatechin (18.3 min).

Regarding the characterization and quantification of methylxanthines corresponding to reading at wavelengths of 270 and 280 nm, the following were detected: (1) theobromine, (2) catechin, (3) theophylline, (4) caffeine and (5) epicatechin, respectively (Figure 01).

Figure 01. HPCL chromatogram referring to the identification of theobromine, catechin, theophylline, caffeine and epicatechin. Source: authors themselves.

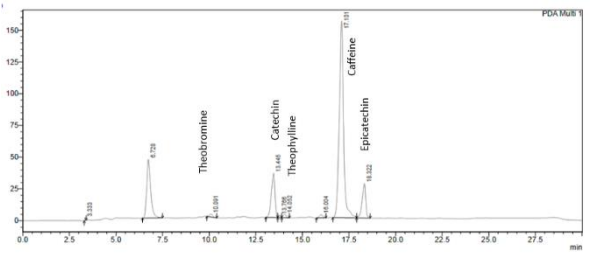


Table 02 describes the quantification values for the markers caffeine, catechin, epicatechin, theobromine and theophylline. It is worth noting

the high levels of caffeine (40.19mg / g) and catechin (42.43 gmg) demonstrating the potential of methylxanthines in the guarana under study.

Table 02. Result of HPLC tests to quantify theobromine, catechin, theophylline, caffeine and epicatechin. Source: authors themselves.

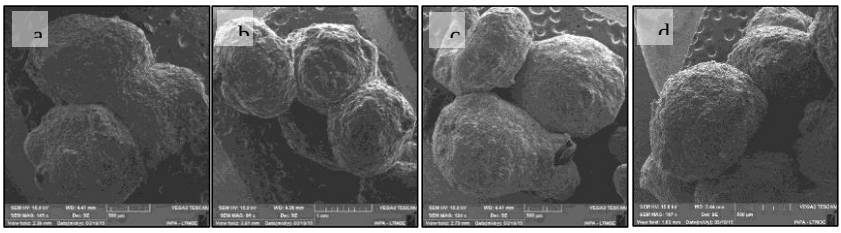
Trateaments	N	Mean (mg/g)	Standar deviation	Minimum	Median	Maxium
Caffeine 270nm	6	40,19	1,11	38,92	39,83	42,12
Catechin 280nm	6	42,43	2,28	38,49	42,63	45,16
Epicatechin	6	20,39	0,56	19,77	20,24	21,36
Theobromine	6	0,25	0,02	0,21	0,25	0,283
Theophylline 270nm	5	0,21	0,03	0,17	0,20	0,251

3.2. Microscopic characterization of the encapsulated powder containing guarana

The drying process of guaraná microcapsules led to the formation of spherical particles of different sizes, characteristic of the powders obtained by microencapsulation. Through Scanning Electron Microscopy (SEM), it is possible to verify the effect of drying in the ionic gelation process on the conformation of the microstructures of guarana extracts.

The results generated by SEM are shown in Figure 02, where granules (Figure 2a, 2b and 2d) with spherical formation were observed, as well as indentations with depressions (Figure 2a, 2b, 2c and 2d) caused by the withdrawal of water during drying after the ionic gelling process.

Figure 02. Micrographs of the microencapsulated guarana extracts: a) Guarana and sodium alginate; b) Guarana, sodium alginate and inulin; c) Guarana, sodium alginate and whein protein; d) Guarana, sodium alginate and maltodextrin. Source: authors themselves.



3.3. Compositions of antioxidant activity

The average results obtained in the HPLC tests were 40.18 mg / g of caffeine, 42.43 mg / g of catechin, 20.38 mg / g of theobromine, 0.25 mg / g of theobromine and 0.21 mg / g theophylline, as shown in Table 02. The guaraná microcapsules were subjected to the determination of the antioxidant capacity of the bioactive compounds of guaraná by the methods of DPPH, ABTS and FT and showed antioxidant activity, according to the values described in Table 03 for treatments T1 (guaraná + sodium alginate), T2 (guaraná + sodium alginate + inulin), T3 (guarana + sodium alginate + whey protein isolate) and T4 (guarana + sodium alginate + maltodextrin).

Table 03: Antioxidant capacity of guarana bioactive in four treatments applied in the DPPH, ABTS and FT methods at a temperature of 35°C. Source: authors themselves.

Treatments	T1	T2	T3	T4
DPPH	1098,5000	1001,0000	1316,0000	1381,0000
	1093,5000	983,5000	1316,0000	1396,0000
	1083,5000	991,0000	1303,5000	1388,5000
Mean	1091,83µM TE	991,83µM TE	1311,83µM TE	1388,50µM TE
Standar Deviation	7,64	8,78	7,22	7,50
ABTS	1127,6667	1134,3333	1474,3333	1577,6667
	1144,3333	1121,0000	1471,0000	1574,3333
	1144,3333	1124,3333	1481,0000	1587,6667
Mean	1138,78 µM TE	1126,56 µM TE	1475,44 µM TE	1579,89µM TE
Standar Deviation	9,62	6,94	5,09	6,94
FT	226,5000	181,5000	234,3571	555,4286
	225,4286	181,8571	232,2143	555,7857
	227,2143	183,2857	234,0000	554,0000
Mean	226,381µM TE	182,214µM TE	233,524µM TE	555,071µM TE
Standar Deviation	0,90	0,95	1,15	0,95

The values obtained by the DPPH method ranged from 983.5 µMTE for Treatment 2 to 1396.0 µMTE for Treatment T4. Similarly, the ABTS and FT methods are observed, in which the lowest values presented are in Treatment T2 (1121.0 µMTE and 181.5 µMTE respectively) and the highest in Treatment T4 (1587.66 µMTE and 555.78 µMTE respectively) .

As for the average, the following results were obtained: T1 = 1091.83 µMTE, T2 = 991.83 µMTE, T3 = 1311.83 µMTE and T4 =

1388.50 µMTE, respectively. In the same way, microcapsules showed good antioxidant activity, with an average value for T1 = 1138.78 µMTE, T2 = 1126.56 µMTE, T3 = 1475.44 µMTE and T4 = 1579.89 µMTE, respectively, using the ABTS method. For the FT method, the mean of the antioxidant activity determination were 226,381 µMTE for T1, 182,214 µMTE for T2, 233,524 µMTE for T3 and 5755,071 µMTE for T4.

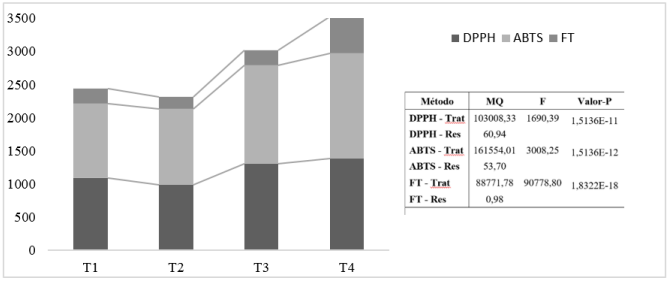
Referring to the comparative statistical data by the analysis of variance "Anova - Single factor", Figure 03 presents the average of the values referring to Treatments T1 to T4 in the three methods used in this work: DPPH, ABTS and FT.

It was possible to observe the values of the active principle between the groups of 99% and only 1% of residue for all methods, however, using a significance level of 0.05 (confidence interval) it is noted that there is at least one treatment different, appearing in the rejection region (P-value ≤ 0.05), where the differences between some of the averages are statistically significant for each method applied.

In this sense, the Tukey test was applied, verifying that the minimum significant difference, for DPPH, ABTS and FT were 6.37, 5.98 and 0.807, respectively.

With the due comparisons of the constructed from the significant differences it was analyzed that there was difference in all the treatments applied to the DPPH and FT methods. For the ABTS method it was analyzed that there was no significant difference between the treatments of the T1 and T2 particulate systems, however there was a significant difference between the treatments T3 and T4.

Figure 03. Average values referring to treatments T1 to T4 in ABTS, DPPH and FT methods for analysis of variance. Source: authors themselves.



Método	MQ	F	Valor-P
DPPH - Trat	103008,33	1690,39	1,5136E-11
DPPH - Res	60,94		
ABTS - Trat	161554,01	3008,25	1,5136E-12
ABTS - Res	53,70		
FT - Trat	88771,78	90778,80	1,8322E-18
FT - Res	0,98		

4. DISCUSSION

The use of High Efficiency Liquid Chromatography shown in Figure 01 and Table 02 is justified by allowing the simultaneous quantification of chemical markers within a reasonable time and at a non-prohibitive cost. The elution conditions of the markers were developed and optimized by adjusting the gradient range of the chromatographic parameters, slope, flow rate and temperature to produce peaks within Rs> 2 for all evaluated markers, as proposed by (Machado et al., 2018).

As eluents of the mobile phase of HPLC, in the present work water and methanol were used. According to the literature, the most used solvents for the mobile phase in a reverse phase HPLC are mixtures of water and acetonitrile or water and methanol, with acetonitrile being the preferred organic modifier. In Lee's work, the analyzes were performed in the wavelength range of 200 to 400 nm and observed in k 220 nm (Lee, 2011).

Applying this same methodology to commercially obtained guarana powder, Bittencourt et al., (2014) obtained the following compositions: caffeine (34.19 ± 1.26mg / g), theobromine (0.14 ± 0.01mg / g) , catechin (3.76 ± 0.12 mg / g), and epicatechin (4.05 ± 0.16 mg / g), the lower compositions being those obtained in this study for all detected markers. In Brazilian commercial samples, contents of approximately 5.0 mg / g were found for catechin (~ 0.46%) and epicatechin (~ 0.55%) using the HPLC-UV method (Machado et al., 2018).

The results of this work indicated that powdered guarana can be a potential source of antioxidants in foods and biological systems. In addition, they indicate a good amount of phenolic compounds in the constitution of guarana de Maués, which can contribute to the antioxidant effect of guarana powder. Guarana seed extracts can be potential natural antioxidants in the food industry and useful for preserving food against a variety of bacterial and fungal species related to food (Majhenic et al., 2007). According to some researchers, a diet rich in antioxidants seems to correlate with a reduced risk of cardiovascular disease, among other beneficial effects (Khramova et al., 2011; Michiels et al., 2012; Salman et al., 2008).

Rezende et al., (2018) obtained results similar to the description in Figure 02 when evaluating the morphology of the acerola microcapsules obtained by spray drying, observing that the majority of the samples presented irregular shapes, smooth surface and spherical conformation, characteristic of the produced microparticles by the spray drying process. Tonon et al., (2009) observed the formation of spherical particles in the spray drying of the açaí pulp, reporting that such forms are characteristic of the microparticles produced by this drying system. Dib Taxi et al., (2003) evaluated the spray drying process of camu-camu, also observing the formation of particles of different sizes and spherical shapes.

The major elements found in the microencapsulated guarana extracts under study were: calcium, iron, phosphorus, potassium and sodium, which are essential minerals for balanced nutrition, such as potassium and sodium, which are essential in the proper balance of fluids, metabolic activity, transmissions nerves, muscle contraction, adequate maintenance of blood pressure and elimination of waste from the body (Gharibzahedi & Jafari, 2017).

With the use of the microencapsulation technique, the carrier agents can promote better handling of the final product obtained, providing greater protection against the absorption of moisture from the environment and making it less hygroscopic. The composition of the carrier agent is the main determinant of the microparticles' functional properties and can be used to improve the performance of a particular ingredient (Gharsallaoui et al., 2007). The carriers can also be used to protect against environmental effects (oxygen, light, humidity, etc.), thus facilitating their handling and acceptability in general (Shivhare et al., 2018). Polymers that can be natural or synthetic are generally used (Azeredo, 2005).

Methods used to determine the antioxidant activity of fruits, when applied independently, may not provide safe results due to the complexity of compounds with antioxidant capacity present in these species. With the different types of radicals and the different sites of action, there is hardly a single method capable of safely and precisely representing the true antioxidant activity of a substance (Frankel & Meyer, 2000). In this context, the ABTS, DPPH and FT methodologies

were used to determine the antioxidant capacity of guarana microcapsules.

The purpose of microencapsulation processes is to maintain the physical and chemical characteristics of bioactive compounds during storage, protecting them from oxidative and degradation processes (Silva et al., 2016). In research by Tonon et al. (2010) on the microencapsulation process of açaí juice, it was observed that the Maltodextrin (MD) treatment acted better in the protection of bioactive compounds compared to arabic gum (GA). Were observed in microencapsulated extracts of lyophilized grapes with GA, polydextrose and guar gum lower values in the Treatment with DPPH compared to the natural extract (Kuck et al., 2016).

In this research, the microencapsulated T1 (guarana and sodium alginate) presented the third best ABTS, DPPH and FT retention capacity of the compounds proposed in this study. Alginate is composed of carboxylic acid, functional hydroxyl, glycosidic groups, glycosidic and glycolic bonds and has been used as a wall material for the complex coacervation encapsulation technique. This polysaccharide is also very useful for immobilizing unstable thermal materials, such as omega 3 and phytosterol, by ionic gelation, as it is able to gel at room temperature (Comunian & Favaro-Trindade, 2016).

Thus, the size of the microparticles obtained as a wall material is directly related to the concentration and viscosity of the alginate solution employed, observing that low concentrations favor the reduction of the particle size. However, reduced concentrations also decrease the mechanical strength and stability of the microparticles (Dickinson, 2011).

The microencapsulated T2 (guarana, sodium alginate and inulin) has the fourth best ABTS, DPPH and FT retention capacity of the compounds proposed in this study. Inulin is a naturally linear polysaccharide (Jain et al., 2014) being a carbohydrate considered prebiotic found in many plant roots, such as dandelion, chicory, asparagus or dahlia and in some cases, directly in fruits or vegetables such as : garlic, onion, wheat, artichoke, asparagus, rye and banana (Mensink et al., 2015; Victória de Barros Fernandes et al., 2016).

Among the examples of applications, inulin is used as: low-calorie sweetener, a substance that provides a solid dispersion to

increase the speed of dissolution, an agent to form gel and to increase the viscosity of solutions and as non-digestible fiber or dietary fiber (Victória de Barros Fernandes et al., 2016). Inulin also has the ability to change the composition of the intestinal flora after a short feeding period based on the results of in vitro and human studies (Mensink et al., 2015).

The microencapsulated T3 (guarana, sodium alginate and whein protein) has the third best ABTS, DPPH and FT retention capacity of the compounds proposed in this study. The whey proteins (b-lactoglobulin and a-lactoglobulin) have a negative charge at pH equal to 6.8 found in fresh milk, allowing its use as a wall material in complex coacervation technique, since it can participate in the electrostatic interactions of the same way as other biopolymers. In addition, due to their globular structure, if native to a solution, these proteins provide only low viscosities, even in high concentrations (Comunian & Favaro-Trindade, 2016).

It is also reiterated that whey protein is a suitable polymer to provide necessary functionality when applied as a wall material. It is important to highlight that the induced heat alters the whey protein conformation due to the disulfide bonds, being an essential information to control the functionality of the material (Comunian & Favaro-Trindade, 2016). Proteins isolated from whey are widely used to stabilize emulsions against flocculation and coalescence via electrostatic and / or steric repulsion, since these proteins contain ionic, polar and non-polar regions in their structure (Gomes et al., 2018).

The results point to a greater retention capacity of the microencapsulated T4 (guarana, sodium alginate and maltodextrin) compared to the other microencapsulated. This starch is widely used for the incorporation of food ingredients due to its excellent properties (Carneiro et al., 2013). It has the advantages of good solubility, neutral aroma and flavor, low viscosity in high concentration and good oxidation stability to the core materials (Zhou et al., 2017). However, it shows poor emulsification and film formation properties. It is therefore necessary to combine maltodexodextrin with other materials that have a good emulsifying and film-forming capacity.

Maltodextrins are generally used as a wall material offering advantages as an encapsulating agent, such as relatively low cost,

neutral flavor and aroma, low viscosity, high solids concentrations and good protection against oxidation. However, a limiting factor of this material is its low emulsifying capacity (Carneiro et al., 2013). This material has the ability to form a cover for the core agents, which encapsulate aromas, flavors and bioactive compounds minimizing exposure to oxygen.

Based on the ABTS and FT analysis between the four microcapsules of the experiment, treatments T1 and T2 revealed less release of bioactive substances that can be explained by the sublimation of water during the drying process, linking these substances with water molecules through the pores formed by water crystals during the freezing process (Rezende et al., 2018).

The results in the DPPH test demonstrate that the antioxidant activity was independent of the raw materials and methods used. It is worth emphasizing the importance of using different tests for the safe and conclusive determination of antioxidant activity, since each method has its specificity and acts in a specific place of action (Rezende et al., 2018).

5. CONCLUSION

The results showed a superiority in the levels of caffeine, catechin and epicatechin compared to other data in the literature in relation to other research on high reverse chromatography techniques.

Regarding the antioxidant activity, the values show that there was a superiority of encapsulation of the T4 Treatment compared to other microcapsules for food formulations aiming to protect the bioactive components against external agents, improving the use of substances by the body and consequently its antioxidant benefit. However, further tests are needed to understand the properties of guarana methylxanthines and tannins that act by slowing the oxidative stress process.

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