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Use of Ora-pro-nóbis (*Pereskia aculeata* Miller) mucilage as a wall material in microencapsulation by complex coacervation

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ABSTRACT. Innovative materials for the encapsulation of bioactive compounds are a permanent challenge. In this sense, the purpose of the study was to evaluate the use of Ora-pro-nobis mucilage (MOPN) as a wall material to replace Arabic gum (AG), in microencapsulation by complex coacervation of bocaiuva (*Acrocomia aculeata*) pulp oil. The MOPN was obtained from green leaves, and the pH, color, water activity, humidity, fixed mineral residue, proteins, lipids, and extraction effectiveness were determined. MOPN was employed as a partial or total replacement of AG in the microencapsulation of bocaiuva pulp oil. Dehydrated and wet mucilages were used to emulsion formation. The interaction between biopolymers, the effectiveness, efficiency of microencapsulation, and the morphological characteristics of microcapsules were confirmed. In addition, the bioactive compounds (chlorophyll and β-carotene) and antioxidant activity were evaluated. The microcapsules were produced using moist mucilage. Microcapsules obtained with AG/gelatin/ bocaiuva oil were considered as control samples. MOPN showed brown color and chlorophyll content of 72.44±5.74 mg 100 g^{-1}. The interaction between AG and MOPN in microcapsule formation was proven for the formulations with 25 and 50% MOPN substitution. The effectiveness was 74.83 and 75.22%, and encapsulation efficiency was 79.18 and 74.59%, respectively, verified through optical micrograph. In addition, the microcapsules with 25 and 50% MOPN as wall material showed three times more antioxidant activity against ABTS, and two times more β-carotene compared to the control microcapsule.

Keywords: Non-conventional food plant (NCFPs); carotenoids; chlorophyll; antioxidant activity; encapsulation.

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Introduction

Pereskia aculeata, commonly known as ora-pro-nóbis (OPN) is a non-conventional food plant (NCFP) rich in proteins and minerals, consumed as food and used in folk medicine for the treatment of wounds, inflammation and as an analgesic (Pinto et al., 2015; Porto, Campos, Carreño, & Garcia, 2021). These properties may be associated with the phenolic acids and flavonoids identified in the leaves (Garcia et al., 2019). OPN leaves have biopolymers, mainly made up of galactose and arabinose units that provide functional properties such as gelling, emulsifying and thickening (Lima Júnior et al., 2013). The mucilage extracted from OPN leaves contains galacturonic acid and as a main component the polysaccharide arabinogalactan type I, which is also present in the structure of the arabic gum (Martin, Freitas, Sassaki, Evangelista, & Sierakowski, 2017). Arabinogalactan is a polymer present in the structure of arabic gum. These properties open perspectives for the study of new wall materials, in techniques such as microencapsulation.

Many microencapsulation techniques are used to obtain encapsulated compounds, highlighting the complex coacervation process, mainly for oils, since it allows interaction, in an aqueous environment, between two oppositely charged polymers (proteins and polysaccharides) the possibility of forming, around droplets of the active material, a capsule. However, the efficiency of the process depends on factors such as the pH of the environment, system turbulence, emulsion droplet size, process temperature, ionic strength, wall material, the latter being one of the most relevant (Lima et al., 2019; Timilsena, Okanbi, Khalid, Adhikari, & Barrow, 2019).

In microencapsulation by complex coacervation, AG stands out as the most used as wall material. AG is a great wall material, however, it is imported, so it has high cost and availability problems, as it is produced in regions highly susceptible to unpredictable climatic variations and political conflicts. Thus, considering the

biodiversity of the Brazilian flora, the study of natural polymers as encapsulating agents that can totally or partially replace AG is extremely important, to provide alternative materials, preferably of low cost, low toxicity, wide availability, and biodegradability. In the Mato Grosso do Sul region, there are species of palm trees with fruits rich in bioactive compounds, such as the *Acrocomia aculeata*, a palm tree from tropical regions and from the Brazilian cerrado, commonly known as bocaiuva or macaúba. The oil extracted from the pulp of bocaiuva has a predominance of unsaturated fatty acids and carotenoids, mainly β-carotene (Lescano et al., 2015). The protection of these bioactive substances from microencapsulation by complex coacervation with AG and gelatin and their possible therapeutic action were duly proven by Lescano, Sanjinez-Argandoña, Arruda, Moraes, and Kassuya (2014) and Lescano et al. (2015), indicating potential for pharmaceutical, cosmetic, nutritional and chemical use (Silva, Campos, Borsato, Candido, & Donadon 2018; Silva, Salomão, De Souza, Ansolin, & Tubino, 2018).

Commonly the gelatin is used as a wall material on the complex coacervation because it's a protein, as a natural food-grade polymer with high complexing capacity with many polysaccharides such as pectins and gums (Devi, Sarmah, Khatun, & Maji, 2017).

In this context, the aim of the present study was to propose and elucidate the technological properties of the OPN's mucilage as a innovative material in the formation of bocaiuva pulp oil microcapsules by complex coacervation, in total or partial replacement of the AG.

Material and methods

Leaves of *Pereskia aculeata* (Ora-pro-nóbis – OPN) were manually collected from the medicinal plant garden in Campus II of the Federal University of Grande Dourados, (UFGD) - Mato Grosso do Sul - Brazil, at coordinates 22°11'43.5" S of latitude, 54°56'07.5"W of longitude and altitude of 430 m and it was transported to the Laboratory. The plants exsiccate was deposited in the herbarium of the Federal University of Grande Dourados (UFGD), under number 5226. Gelatin pH EUR (Sigma-Aldrich, São Paulo, Brazil) and AG (Dinâmica, São Paulo, Brazil) were acquired with an analytical degree. Bocaiuva oil (*Acrocomia aculeata*) was extracted by cold pressing from the pulp of the ripe fruit.

Obtaining the Ora-pro-nóbis mucilage

The leaves of Ora-pro-nóbis (OPN) were manually selected in order to obtain an uniform lot in terms of leaf size and color (dark green), with an average size of 14.18±0.23 cm, verified with a caliper (Lotus Plus, China), washed in running water, weighed, packed in polyethylene bags, sealed and stored at -5ºC until use.

The hydrocolloids of fresh OPN leaves were obtained using the method described by Lima Júnior et al. (2013). The frozen green leaves of OPN (1000 g) were crushed in 2500 mL of hot water (~80°C). The crushed material was placed in a laboratory incubator (Te-420, Tecnal, São Paulo, Brazil) with agitation (100 rpm) at 55ºC for 7.5 hours. After that, the material was subjected to two filtrations on organza fabric: one at normal atmospheric pressure and the other under vacuum using a Buchner funnel, obtaining a filtrate and a solid residue. The product obtained on this step was called Filtrate 1. The solid residue was discarded.

Filtrate 1 was subjected to precipitation in 96% ethyl alcohol at a ratio of 3:1 (alcohol: filtrate). The solution was homogenized and left to rest for 24h at 4°C to obtain the precipitation of the mucilage. The precipitation was performed separately, removing the precipitated every 24h, resulting in three repetitions, in order to guarantee the total removal of the mucilage. Finally, the precipitated mucilage was centrifuged at 11,500 rpm for 20 min. in a centrifuge (Hettich, Rotanta 460, Germany), for its complete separation and removal of the remaining alcohol.

The mucilage obtained was divided into two parts in which the wet mucilage was placed in polyethylene bags and stored at -5ºC, and the other part was dehydrated in an oven with air circulation (30°C for 30h). The dried product was ground in an electric mill (Krups, model 208, Hongkong), packed in falcon tubes and stored at -5ºC, obtaining dehydrated mucilage. The wet mucilage was used to produce the microcapsules because the dry mucilage wasn't soluble in water and the dehydrated mucilage was used for the physical-chemical analyses.

Mucilage extraction yield

The OPN mucilage extraction yield was calculated from Equation 1.

$$RMOPN(\%) = \frac{MSMOPN(g)x100}{leaves(ss)} \quad (1)$$

In which, RMOPN is the Ora-pro-nóbis mucilage yield (%), MSMOPN is the dehydrated mucilage (g) and leaves (ss) is the mass of OPN leaves minus moisture (g).

Dehydrated mucilage characterization

Color analysis

Instrumental color analysis was performed using a colorimeter (Minolta, model CR400, Japan) at 25°C, using the CIELAB color system, obtaining the parameters of L*, a* and b*. The L* value expresses brightness and ranges from 0 (black) to 100 (white). A* values indicate chromaticity ranging from green (-) to red (+) and b* values range from blue (-) to yellow (+). From these parameters, the color saturation (C*) and color tone (h°) were calculated, based on equations 2 and 3, respectively.

$$C^* = \sqrt{a^2 + b^2} \tag{2}$$

$$h^\circ = arctan\frac{b^*}{a^*} \tag{3}$$

pH and water activity

The determination of pH was performed by direct measurement (Method nº 981.12, Association of Official Agricultural Chemists [AOAC], 1997), in a digital pH meter (Tec-7, Tecnal, São Paulo, Brazil). Water activity was determined in an Aqualab hygrometer (Pre-dew Decagon Devices Inc, USA), at 25ºC, previously calibrated with distilled water (A_w = 1,000).

Chemical analysis

Moisture contents were determined in an oven with air circulation at 70ºC for 24h (Method Nº 44-15.02, American Association of Cereal Chemists [AACC], 2010); mineral residue fixed in a muffle furnace at 550°C (Method Nº 08-01.01, AACC, 2010); proteins by the Kjeldahl procedure (Method Nº 960.52, AOAC 1997) and total lipids by the cold Bligh-Dyer method (Method Nº 920.39, AOAC, 1997). The fiber content was quantified by an acid and alkaline hydrolysis (Method 985.29, AOAC, 1997). Titratable acidity was determined by neutralization volume, expressed in g of citric acid 100 g^{-1} of sample (Method Nº 942.15, AOAC 1997).

Microencapsulation by complex coacervation

The concentration of encapsulating agents and operating conditions of the microencapsulation method were performed as described by Lescano et al. (2014), with some modifications. Five formulations were prepared according to Table 1, in which A was the control formulation, obtained with bocaiuva oil (5%) and wall materials: gelatin (5%) and AG (5%). In formulations B, C, D and E, the AG content was partially or totally replaced by the MOPN. The amount of MOPN incorporated in each formulation was determined considering the total solids present in the wet mucilage.

Because it's a protein, as a natural food-grade polymer with high complexing capacity with many polysaccharides such as pectins and gums. The gelatin is commonly used as a wall material on the complex coacervation (Devi et al., 2017). Therefore, on this study the control formulation was composed with gelatin and arabic gum. The gelatin was used in all formulations, on the fixed amount of 5% varying the quantity of AG and MOPN.

Table 1. AG and MOPN concentration for the preparation of bocaiuva pulp oil microcapsules by complex coacervation.

Formulation	Gelatin (%)	AG (%)	MOPN (%)
FA	5.00	5.00	0.00
FB	5.00	3.75	1.25
FC	5.00	2.50	2.50
FD	5.00	1.25	3.75
FE	5.00	0.00	5.00

AG: Arabic gum. MOPN: Ora-pro-nobis mucilage. FA (control): 100% AG.
FB:75% AG and 25% MOPN. FC 50% AG and 50% MOPN. FD 100% MOPN

The emulsion (O/W) was formed by mixing the bocaiuva oil (1 g) in 20 mL of gelatin solution (5%) heated (60ºC), under stirring in the Ultra-Turrax at 18000 rpm for 1 min. Then, the emulsion was placed in a magnetic

stirrer/heater at 1100 rpm (RT 10, IKA, Germany) incorporating 20 mL of AG (5%) or wet MOPN (according to Table 1) and 80 mL of deionized water, both previously heated to 60°C.

Then, the pH was corrected with hydrochloric acid (0.1 M) until reached pH 4.0. The material was placed in an ice bath under constant agitation in a magnetic stirrer (CienlaB, São Paulo, Brazil) at 1000 rpm to reach 10ºC, then stored in a refrigerator at 10ºC, ± 2ºC, for 24h, obtaining the wet microcapsules, by sedimentation. A part of the wet microparticles was destined for analysis and another part was frozen at -60°C and later, lyophilized at -50°C with a vacuum pressure lower than 130 mmHg in the lyophilizer (Liotop, Liobras, Brazil).

Characterization of microcapsules

Optical microscopy

The wet microcapsules were examined under an optical microscope (Nikon Eclipse – 200), equipped with Motic Image Plus software to obtain the images. The observation and capture of images were performed using a magnification with a 40x objective.

Scanning electron microscopy

The dried microcapsules (lyophilized) were observed by scanning electron microscopy (SEM) under the microscope (ZEISS, EVO, LS 15, Germany), the equipment operated at 10 kV and the images chosen were magnified at 300 and 1000x.

Encapsulation performance and efficiency

The yield was calculated from the separation of the phases (supernatant and sediment) during the microcapsule formation process (Lescano et al., 2014). The microcapsules were sieved in a mesh with an opening of 0.075 mm for total separation of the supernatant and sediment (microcapsules). The sediment was dried in an oven at 70ºC for 24 hours and the dry mass was determined. Afterwards, the yield was calculated (Equation 4).

$$R(\%) = \frac{MSP}{MSI} x100 \quad (4)$$

In which, R stands for the sediment's yield (%), MSP is the dry sediment's mass (g) and MSI is the total mass of encapsulating polymers + bocaiuva oil (g).

The microencapsulation efficiency (ME) was determined by the amount of oil present in one gram of microcapsules, in relation to the amount of oil initially inserted in the microencapsulation process, according to the Bligh and Dyer method, with some adaptations according to Equation 5 (Lescano et al., 2014).

$$EM(\%) = \frac{OEM}{OIM} x100 \quad (5)$$

In which, EM stands for the microencapsulation efficiency, OEM is the mass of encapsulated bocaiuva oil (g) and OIM is the mass of oil added in the emulsion (g) per gram of polymers.

Bioactive compounds

Carotenoids

The carotenoids were determined according to the method described by Rodriguez-Amaya (2010), an aliquot of the sample (2 g of OPN leaves, 4 g of MOPN, 0.5 g of bocaiuva pulp oil or 1.5 g of lyophilized microcapsules) was macerated in a mortar with 50 mL of acetone at 10ºC for extraction. of carotenoids. The extracts were evaluated in a spectrophotometer with a wavelength of 450 nm. Petroleum ether was used as white. The total carotenoids (Ct) were calculated from equation 6 and the results were expressed in µg/g of the sample, on a dry basis.

$$Ct\left(\frac{\mu g}{g}\right) = \frac{Abs\ x\ V\ x\ 10^4}{E_{1cm}1\%\ x\ M_{sample}} \quad (6)$$

In which, Abs stands for the absorbance at maximum absorption peak, V is the final sample volume (mL), m_{sample} is the sample mass (g), $E_{1cm}^{1\%}$ is the extinction coefficient (β- carotene = 2592) in petroleum ether (Rodriguez-Amaya, 2010).

Chlorophyll

The percentage of chlorophyll present in OPN leaves and MOPN was determined according to the method described by Lichtenthaler (1987). An aliquot (1.5 g) of the sample was ground with celite in a mortar with 10 mL of acetone solution (80%) (v/v) until the color disappeared. Aliquots of the obtained extract were submitted to absorbance reading in a UV-VIS spectrophotometer (Biochrom, Libra model S60PC) at wavelengths of 647 and 663 nm. Pure acetone was used as white. Total chlorophyll was calculated using Equations 7, 8 and 9. Results were expressed in mg of chlorophyll per 100 g of sample, on a dry basis.

$$Chlorophyll\ a = 12{,}25\ A_{663} - 2{,}79\ A_{647}\ x\ V_{acet} M_{Am}\ x\ dilution \quad (7)$$

$$Chlorophyll\ b = 21{,}50\ A_{647} - 5{,}10\ A_{663}\ x\ V_{acet} M_{Am}\ x\ dilution \quad (8)$$

$$Total\ chlorophyll = 7{,}15\ (A_{663}) + 18{,}71\ (A_{647})\ x\ \frac{V_{acet}}{M_{Am}}\ x\ dilution \quad (9)$$

In which, A_{663} and A_{647} are the absorbances at their respective wavelengths. V_{acet} is the volume of acetone (mL), M_{Am} stands for the sample's mass (g).

Antioxidant activity

The extract was obtained from an aliquot of sample (2.5 g of OPN wet mucilage from leaves or microcapsules and 1 g of bocaiuva oil) mixed with 40 mL of acetone (99%), homogenized, and left to rest for 60 min. at 25ºC, then centrifuged at 11500 rpm for 20 minutes. The recipient's supernatant was transferred to a balloon (100 mL) and the process was repeated with the residue. The supernatants from the two extractions were mixed with distilled water and made up to volume, until it completed 100 mL, this being the extract.

The ability to extinguish free radicals of $ABTS^{+}$ was evaluated by the method proposed by Rufino et al. (2010). Absorbance readings were taken at 734 nm. A standard curve was prepared using Trolox at concentrations of 100, 500, 1000, 1500 and 2000 µM. (R^2 of 0.9932 of the line's equation). Percent inhibition was calculated in relation to the Trolox standard curve. Results were expressed as µM Trolox g^{-1} sample, on a dry basis. The spectrophotometer was previously calibrated with 99% ethanol.

The determination of antioxidant activity by the DPPH method was performed according to the protocol of the Department of Basic Sciences – USP (University of São Paulo), FZEA (University of Animal Husbandry and Food Engineering). An aliquot of 0.2 mL of the sample, extracted previously diluted in acetone (99%), and 1.8 mL of DPPH + a Methanolic solution (0.5 mM) were mixed and after 180 min. of incubation at 25ºC the absorbance was read at 515 nm using a spectrophotometer previously zeroed with methanol.

The same procedure for the samples was performed for the positive control (BHT). Results were expressed as a percentage of DPPH's radical scavenging activity.

$$AS(\%) = \frac{Abs_0 - Abs_{sample}}{Abs_0}\ x100 \quad (10)$$

In which AS stands for the DPPH's radical scavenging activity (%), Abs_o e Abs_{sample} are the absorbances of the control and the sample.

Statistical analysis

All analyzes were performed in triplicate and the microencapsulation experimental tests were repeated seven consecutive times. The results were stated as the average's calculation of the repetitions, standard deviation, and coefficient of variation. The yield and efficiency values of microencapsulation, chlorophyll and carotenoids were subjected to the analysis of variance and test of significant difference ($p < 0.05$) of averages by Tukey, with the aid of the *Statistica* version 8.0's program.

Results and discussion

Ora-pro-nóbis Mucilage

The mucilage extracted from fresh leaves of MOPN presented a dark gelatinous appearance, soluble in water and alcohol, obtaining good hydration and solubilization. The dehydrated MOPN was insoluble in water, observed by the decanting of the material. Probably during drying, there was an irreversible collapse

of the biopolymer structure. In view of this conclusion, the mucilage used as a wall material, in the formation of microcapsules, was the wet MOPN.

The MOPN's yield in relation to the OPN leaves was 14.73%, on a dry basis. It is known that the water content presented in the leaves can vary, influencing the extraction yield, as well as the water contained in the extracted mucilage, therefore it is recommended to consider the material on a dry basis. Martin et al. (2017), on the mucilage extraction study for the same species obtained the same mucilage yield of 5.4 and 1.6% for the clarified extracted from the green leaves, but the author didn't consider the humidity present on the leaves and extracted mucilage.

Mucilage Characterization

The results of the color parameters, water activity and pH of the dehydrated MOPN mucilage are presented in Table 2. Considering the color parameters, the values obtained for the mucilage showed a dark color (L* < 50), with a slightly yellow pigmentation prevailing (b* > a*). The value of the chroma (C*) or color intensity was similar to the value of b* (7.17), which represents a slight yellow color that combined with the value of L*, on the color spectrum reflects the brown color, confirmed by visual observation. The color change in the mucilage was already expected, since the leaves used for the extraction of the mucilage went through successive process steps such as cutting, grinding, agitation, heating, drying, causing the oxidation of the thermosensitive constituents, changing the color from green to brown (Silva, Amaral, Junqueira, Leite, & Resende, 2017).

Regarding the water activity, the dehydrated MOPN showed low water activity (0.324), under these conditions the material is stable and microbial growth hardly occurs, provided it is stowed in an appropriate package. Regarding the pH, the values obtained are close to the value of neutrality (6.55). The pH is an important condition for the material to be considered suitable as a wall material. In the pH range (6.0 – 8.0) the interactions between the biopolymers and the water molecules are favored, increasing the viscosity of the environment, under these conditions it is not possible to see the formation of coacervate. However, in the pH range of 4.0 – 5.0 the forces of attraction between the biopolymers (charged by opposite charges) are favored, leading to the formation of the insoluble complex, observing a phase separation. Therefore, in this pH range, the maximum yield of coacervation usually occurs (Yücetepe et al., 2021).

Table 2. Color parameters, water activity and pH dehydrated ora-pro-nóbis' mucilage (MOPN)

Analysis	Value
Color: L*	26.81 ± 0.36
a*	0.69 ± 0.15
b*	7.17 ± 0.36
C*	7.20 ± 0.37
h°	1.48 ± 0.02
Water activity	0.324 ± 0.00
pH	6.55 ± 0.04

Average data from the calculation of the three replicates ± standard deviation. SD. L*, clarity. a*, values indicate chromaticity ranging from green (-) to red (+) and b* values range from blue (-) to yellow (+), C*, color saturation and h°, color tone.

The results of the chemical composition of MOPN are presented in Table 3. The low humidity of the dehydrated mucilage (6.67 g 100 g^{-1}) corroborates with the water activity (0.324), maintaining stability at room temperature. However, the low humidity may have influenced the solubility of MOPN, since it was not soluble in water. The water percentage of the wet mucilage was 68.64%.

Table 3. Chemical composition of dehydrated Ora-pro-nóbis mucilage

Constituint	Value
Humidity (g 100 g^{-1})	6.67 ± 0.09
Proteins (g 100 g^{-1})	13.96 ± 0.01
Lipíds (g 100 g^{-1})	4.21 ± 0.11
Fixed mineral residue (g 100 g^{-1})	7.89 ± 0.01
Fibers (g 100 g^{-1})	2.09 ± 0.17

The values represent the average ± SD (standard deviation) of three repetitions.
Values calculated on a dry basis.

The protein content (13.96 g 100 g^{-1}) was like the one reported by Junqueira, Amaral, Félix, Prado, and De Resende (2019), the authors obtained 10.47% of protein in the powdered MOPN, which had a moisture content

of 13.45%, which represents 0.12 g of protein per gram of sample on a dry basis. In the present study, considering the moisture content, the protein content was 0.15 g g^{-1} of sample on a dry basis.

The lipid content (4.21 g 100 g^{-1}) resembled the one found by Rodrigues, Marinelli, Otoboni, Tanaka, and Oliveira (2015), for OPN leaf flour (4.01 g 100 g^{-1}) containing 12 .89% moisture. The fiber content in the mucilage is much lower compared to the OPN flour (31.40%), indicated by the authors, given that mucilage is a hydrocolloid without insoluble fibers.

Microencapsulation

The emulsions formed from the control formulation (A) and the formulations with partial replacement of AG by MOPN (B, C, D and E), are shown in Figure 1.

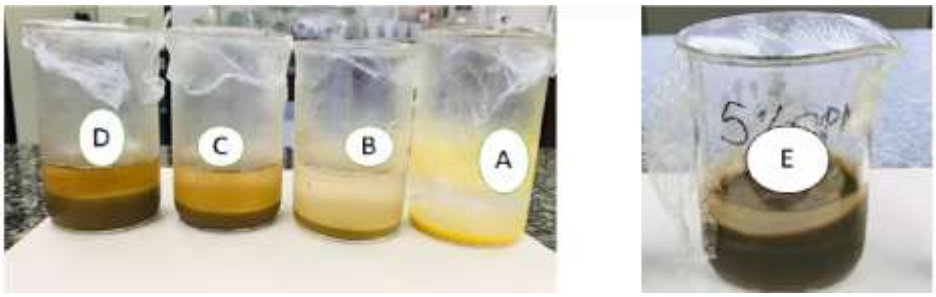

Figure 1. Phase separation of microcapsules from the control formulation (FA), from formulations with partial replacement AG by MOPN, (FB, FC and FD) and formulation E. Formulations (FA): AG 100% (control). (FB): AG 75% and 25% MOPN. (FC): AG 50% and 50% MOPN. (FD): AG 25% and 75% MOPN. (FE) MOPN 100%.

Complete sedimentation of the microcapsules is observed, after the cooling stage, in formulations A and B. Formulation C presented sedimentation, but there was turbidity in the aqueous phase and in formulation D, the turbidity was higher. In the formulation containing 100% MOPN (total replacement of AG) there was no sedimentation or phase separation, the even gelatinous aspect remained in the system, therefore, formulations D and E were discarded.

Microencapsulation's yield and efficiency

The results of microencapsulation's yield and efficiency in microcapsule formation arein Table 4. The yield of microcapsules (~75%) formed with partial replacement of AG by 25% (Formulation B) and 50% (Formulation C) were similar to the control formulation (Formulation A). However, the yield is related to the microcapsule formation and not to the oil encapsulation, since the encapsulation efficiency was significantly higher ($p < 0.05$) in the control formulation (A), followed by the B formulation (50% MOPN), which presented 79.1 ± 0.97% efficiency.

Lescano et al. (2014) defined the best microencapsulation conditions by complex coacervation for the bocaiuva oil (5g of filling, temperature of 60ºC and homogenization speed of 18000 rpm), obtaining yield and efficiency of 95.89 and 97.86%, respectively. Values higher than those found in the present study, these differences can be attributed to the molecular interaction of AG with MOPN during the emulsion, making it necessary to study improvements in the process conditions to increase the efficiency of the interaction between the polysaccharides, such as a longer time of emulsion, which may favor an increase in encapsulation yield and efficiency.

Table 4. Microencapsulation's yield and efficiency of bocaiuva pulp oil using control formulation (A) and different concentrations of MOPN (B to E) as wall material

Formulation	Encapsulation yield (%)	Encapsulation efficiency (%)
A	74.78 ± 2.14[a]	83.06 ± 0.87[a]
B	74.83 ± 2.13[a]	79.1 ± 0.95[b]
C	75.22 ± 1.97[a]	74.59 ± 0.97[c]
D	*	*
E	*	*

Different letters in the same column indicate a significant difference ($p < 0,05$) between the samples.
Formulations (FA): AG 100% (controle). (FB): AG 75% and 25% MOPN. (FC): AG 50% and 50% MOPN. (FD): AG 25% and 75% MOPN. (FE) 100% MOPN.
*There was no formation of microcapsules.

ther studies with Brazilian cerrado fruit oils using AG associated with gelatin as a wall material obtained a yield of 96.45% and an efficiency of 96.68% in the complex coacervation of pequi oil (Justi, Sanjinez-Argandoña, & Macedo, 2018); and 82.55 and 70.72% of yield and efficiency, respectively, for bacuri oil (Lima et al., 2019). These studies suggest that the results obtained in the present work are considered effective, since the efficiency is within the values obtained by these authors.

Microcapsules Characterization

Morphology

Figure 2 illustrates the morphology of the bocaiuva oil microcapsules. Samples A (control), B (25% MOPN) and C (50% MOPN), were presented as a reservatory in which the core was surrounded by the wall material. The rounded and/or oval shape, multinucleated, defined walls protecting the nucleus were also observed.

In formulation D (75% MOPN) dispersed microcapsules and particles of oil and/or encapsulating agents were observed, given that formulation D did not show complete sedimentation of the microcapsules, obtaining three phases: MOPN, dark in color, gelatin more light-colored, AG and bocaiuva oil as supernatant, which justifies the observed morphology.

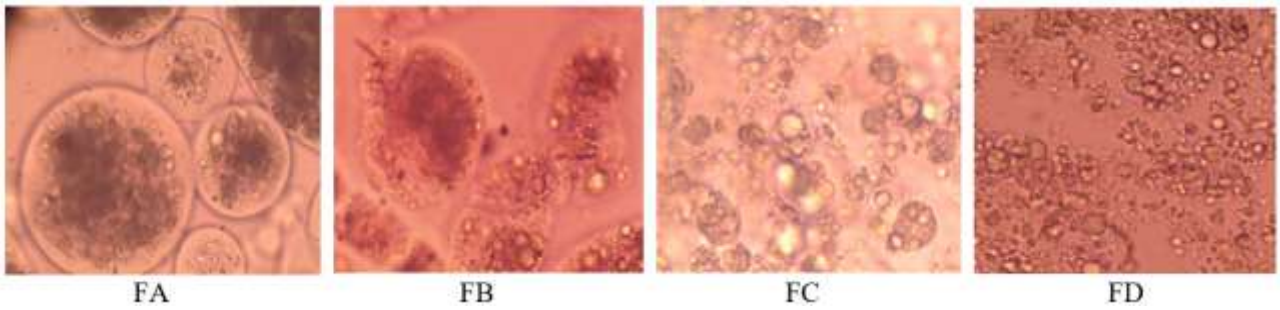

Figure 2. Micrograph of wet microcapsules obtained by optical microscopy of formulations A (100% AG), B (75% AG + 25% MOPN), C (50% AG + 50% MOPN) and D (25% AG + 75% MOPN). Object-glass of 40 x.

Scanning electron microscopy

The micrographs obtained by scanning electron microscopy of the microcapsules are shown in Figure 3. All the micrographs showed irregular shapes and varied sizes and resembled the flake structure, characteristic of powders obtained by the lyophilization process. Despite the observed agglomeration behavior, the microcapsules remained stable, that is, there was no rupture of the walls (Rajam & Anandharamakrishnan, 2015).

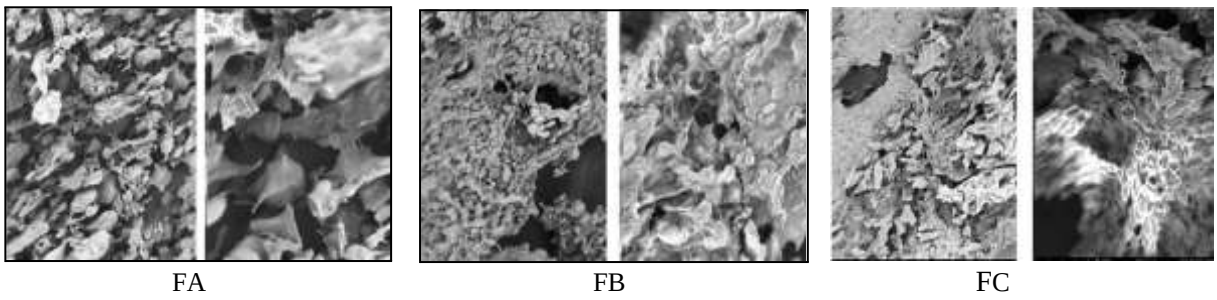

Figure 3. Micrograph of microcapsules obtained by scanning electron microscopy of formulations A (100% AG), B (75% AG + 25% MOPN) and C (50% AG + 50% MOPN). Each image from left to right has an amplification of 300 x and 1000 x, respectively.

The presence of microcavities and pores on their surfaces was also observed in samples of microcapsules of ginger essential oil microencapsulated by complex coacervation using the following as wall material: whey protein/AG and AG/chitosan. The authors attributed the observed aspect to the lower formation of ice crystals in the freezing step of the coacervate, prior to lyophilization, leading to the formation of a porous structure (Tavares & Noreña, 2020).

Comunian et al. (2013) produced microcapsules by complex coacervation and described the lyophilized coacervates as agglomerated and had solid bridges connecting the ascorbic acid microcapsules, which is typical of coacervate microcapsules that were subsequently lyophilized. In the present study, the irregular shapes can also be explained by the presence of soluble fibers in the mucilage (Martin et al., 2017).

Bioactive compounds

Chlorophyll and β- carotene

Table 5 presents the results of the analyzed bioactive compounds. The fresh OPN leaf showed high levels of chlorophyll a, b and total. In mucilage, the total chlorophyll content represents 12.81% of the chlorophyll content found in the leaf, despite its degradation with heat treatment during the extraction process. During storage, under freezing, it's seen that the green color of chlorophyll *a* turn into a brown color, attributed to the formation of pheophytin (Heaton, Lencki, & Marangoni, 1996).

Table 5. Bioactive compounds: Chlorophyll a, b and total contents in leaves and mucilage of *P. aculeata* (OPN) and β-carotene in leaves and mucilage of OPN, oil and lyophilized microcapsules of bocaiuva pulp oil

Bioactive compounds	Leaves	Mucilage	Bocaiuva Oil	Microcapsules		
				FA	FB	FC
Chlorophyll a (mg 100 g^{-1})	380.2 ± 17.93[a]	53.39 ± 5.00[b]	na	na	na	na
Chlorophyll b (mg 100g^{-1})	185.09 ± 6.11[a]	19.06 ± 0.79[b]	na	na	na	na
Total Chlorophyll (mg 100 g^{-1})	565.32 ± 24.04[a]	72.44 ± 5.74[b]	na	na	na	na
β- carotene (µg g^{-1})	479.61 ± 8.54[b]	556.80 ± 4.46 [a]	466.42 ±4.19[c]	112.67 ± 0.92[f]	177.32 ± 1.35[e]	226.45 ± 0.97 [d]

Different letters in the same column indicate a significant difference ($p < 0,05$) between the samples. Formulations FA (control, 100% AG), FB (75% AG and 25% MOPN) and FC (50% AG and 50% MOPN). Results expressed on a dry basis, indicate average ± standard deviation. Na, not applicable.

Regarding the β-carotene, the values found for its leaves are higher than those reported by Almeida, Junqueira, Simão, and Corrêa (2014), who reported a β-carotene content of 24.07 ± 0.37 mg 100 g^{-1} for leaves of *P. aculeata.* In this study, the β-carotene value for OPN leaves was 47.96 ± 8.54 mg 100 g^{-1}.

Among the bocaiuva oil microcapsules, those formulated with MOPN had a higher β-carotene content. The microcapsule of formulation FC presented 2.2 times more β-carotene than the control formulation (FA), and formulation FB indicated 1.5 times more β-carotene compared to the control.

The conservation of carotenoids presents in oils extracted from brazilian cerrado fruits and microencapsulated by complex coacervation was also verified by other authors. Justi et al. (2018), in pequi oil microcapsules, obtained total carotenoid ranging from 60.30 to 192.04 µg g^{-1}. France et al. (2019), in the analysis of macauba oil, obtained 267.37 ± 3.09 mg of β-carotene 100 g^{-1} of oil. Lima et al. (2019), in bacuri oil microcapsules, showed carotenoids of 67.83 and 77.90 µg g^{-1}, highlighting the efficiency of microencapsulation by complex coacervation.

Antioxidant capacity

The results of the antioxidant activity of the microcapsules, the oil, the OPN and MOPN leaves and the BHT (Butyl-Hydroxytoluene) control are shown in Table 6. The microcapsules of formulations FB (25% MOPN) and FC (50% MOPN) showed higher antioxidant activity by both methods, when compared to the control formulation, with the FB formulation presenting approximately 2.9 times and the FC formulation 3.44 times more antioxidant activity in relation to the FA control in the results obtained by ABTS +.

In the present study, bocaiuva oil stored for approximately 12 months under refrigeration was used, probably the storage time caused the degradation of the bioactive compounds, which justifies the value of the lower antioxidant capacity of the bocaiuva oil used (Table 6). However, most oils are sensitive to oxidative deterioration due to storage time, and fatty acid oxidation may occur when exposed to temperature, light, humidity, and oxygen with a loss of quality (Berasategi, Barriuso, Ansorena, & Astiasarán, 2012; Evaristo et al., 2016).

The pure bocaiuva oil showed lower antioxidant activity against the FB and FC microcapsules, and higher against the control microcapsule. This can be explained by the high antioxidant activity of MOPN. The values of the microcapsules of the FB and FC formulations were higher, observing the influence of mucilage in the microencapsulation system.

The results obtained for the percentage of the radical scavenging activity of DPPH• showed the antioxidant action of 90.95% of BHT, which was expected considering that BHT is one of the most used synthetic antioxidants in the food area, however, microcapsules and oil showed low activity. Some researchers report that the method using DPPH• is efficient in hydrophilic materials, not being effective in hydrophobic materials. Thus, these results show the importance of choosing the method.

Table 6. Microcapsules antioxidant activity by coacervation, MOPN and bocaiuva pulp oil

Sample	$ABTS^{+}$ (µM Trolox g^{-1} $_{sample}$)	Radical scavenging activity of DPPH • (%)
OPN leaves	9532.86	nd
Mucilage	2869.61	8.11
Bocaiuva oil	612.56	9.46
Microcapsule FA	363.18	1.35
Microcapsule FB	1046.06	2.70
Microcapsule FC	1252.49	2.70
BHT	24448.90	90.95

*Results expressed on a dry basis.

Conclusion

Ora-pro-nóbis mucilage (MOPN) exhibited a gel-like texture and exceeded reported yields. Combining MOPN with AG efficiently generated bocaiuva oil microcapsules using complex coacervation. Substituting 25-50% of AG with OPN mucilage as the wall material yielded microcapsules with >74% encapsulation efficiency, carrying 2.2 times more β-carotene than AG-based counterparts (control). In terms of antioxidant activity (ABTS+), MOPN-infused microcapsules, especially the FC formulation, demonstrated approximately 2.9 to 3.44 times greater activity than the FA control. These promising findings indicate that MOPN holds potential as a microencapsulation material for probiotics, vitamin C, and omega-3 in food and nutraceutical applications.

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