

Extraction, microencapsulation, and application of anthocyanins from juçara palm fruit (*Euterpe edulis* Mart.): enhancement of natural pigment

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Abstract The Juçara fruit (*Euterpe edulis* Martius) has been progressively standing out for presenting significant biological and nutritional activity. Its functional characteristics are related to its high content of anthocyanins, which, when isolated, are highly unstable, limiting their applications. The present research proposed to obtain an anthocyanin-rich extract from the juçara pulp, microencapsulate it with the maltodextrin and beta-cyclodextrin (beta-CD) matrices and evaluate the stability of the microencapsulated anthocyanins against light, pH, and milk development fermented. The use of encapsulating agents brought the anthocyanins significant thermal and light stability, in addition to intensifying their colors in a broader pH range. The FTIR-ATR techniques and the thermal analyzes of DSC and TGA showed that there was no molecular inclusion between the anthocyanins in the extract and beta-CD, but there was a physical interaction with the maltodextrin. In the development of fermented milk, the use of maltodextrin showed better product color stability. Therefore, anthocyanin microencapsulation processes can contribute to the development

of innovative, more stable, and effective commercial food products.

Keywords Phenolic compounds · Functional ingredient · Antioxidant activity · Natural dye · Microencapsulation

Abbreviation

Beta-CD Beta Cyclodextrin

Introduction

Consumers' search for foods that provide health benefits, in addition to nutritional and sensory quality, has grown significantly. This has motivated the food industries to invest significantly in the development of innovative research and technological solutions that involve the segment of food or functional ingredients (Bicudo et al. 2014; Martins et al. 2020).

The juçara (*Euterpe edulis* Mart.), popularly known as açai from the Atlantic Forest or just juçai, is widely found in the South and Southeast regions of Brazil and is gaining prominence for presenting intense biological activity and high nutritional values (Schulz et al. 2016). The fruit has functional characteristics because it has high levels of anthocyanins and other phenolic compounds, which give it antioxidant activity, in addition to unsaturated fatty acids, especially oleic and linoleic (Carpiné et al. 2020).

Anthocyanins are red–purple flavonoid pigments responsible for a wide variety of colors in fruits and flowers (Schulz et al. 2016). These compounds have high applicability in the food industry because they present strong pigmentation and high solubility in water, being a promising alternative to artificial colors, which have been replaced worldwide, due

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to the various scientific studies on their harmful potential (Bicudo et al. 2014; Zhang et al. 2020).

Due to their antioxidant power, anthocyanins have protective and health-promoting actions (Tarone et al. 2020). However, when isolated, they are highly unstable to environmental and process factors, such as pH, light, and heat, and very susceptible to degradation, which makes their industrial application challenging. To enable the application of these compounds in the development of new food products, the use of microencapsulation and molecular inclusion techniques has been explored (Mangolim et al. 2014). These techniques allow improving the physical–chemical properties of the materials, in addition to providing greater stability and protection of bioactive compounds during the development and storage of food (Torone et al. 2020).

The innovative formatting is combined to solve as instability as possible the possibility of lyophilization and molecular inclusion techniques, which improves the bioinclusion of anthocyanins (Milea et al. 2020). It is also possible to find innovative and emerging encapsulation technologies in the literature, such as: eletospraying, nano spray drying, eletrostatic spray drying, nanoencapsulation, among others (Veneranda et al. 2018; Zaeim et al. 2019; Jayaprakash et al. 2022).

Several microencapsulating agents have been used to stabilize substances, such as maltodextrin and cyclodextrins (CDs). Maltodextrin is a complex carbohydrate from starch, consisting of glucose polymers, while CDs, also made up of glucose units, are cyclical and liable to form an inclusion complex with a variety of molecules.

Given the above, the present study aimed to obtain an extract rich in anthocyanins from the pulp of juçara palm fruit (*Euterpe edulis* Mart.), microencapsulate it with the maltodextrin and beta-cyclodextrin (beta-CD) matrices and evaluate the stability of microencapsulated anthocyanins against light, pH and the development of fermented milk. The FTIR-ATR techniques and the thermal analysis of DSC and TGA were used to study molecular inclusion, and spectrophotometry was employed to evaluate the stability of microencapsulated anthocyanins.

Materials and methods

Materials

The juçara pulp was purchased frozen from ASPRAN—Association of Small Rural and Artisanal Producers of Antonina (Antonina-PR-Brasil). Beta-cyclodextrin (beta-CD) (99.5% purity) was purchased from Sigma Chemical Company and maltodextrin (DE 20) from CornProducts Brazil. The other reagents were of analytical grade. The reagentes 2,2'-azino-bis 91 (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and

2,2-diphenyl-1-picrylhydrazyl 92 (DPPH), were purchased from Sigma-Aldrich Brazil Ltda.

Obtaining anthocyanin extract from juçara pulp

Anthocyanins were extracted according to the methodology proposed by Passos et al (2015). A ratio of 1:2 (wt/wt) of the fruit pulp with a solution of ethanol and water in the proportion 7:3 (v/v) was used. The mixture was subjected to stirring on a magnetic stirrer for 40 min protected from light. After extraction, the mixture was vacuum filtered and concentrated to 30% of the initial volume in a rotary evaporator (at 45 °C and protected from light). The obtained extract was frozen, lyophilized at – 50 °C for 48 h, and stored at – 15 °C in amber glass bottles.

Dosage of total anthocyanins, phenolic compounds, and antioxidant activity of the extract

The content of total anthocyanins in the lyophilized extract was determined by the differential pH method described by Lee et al (2005), in which two buffer systems are used, one being potassium chloride pH 1.0 and 0.025 mol/L and the second sodium acetate pH 4.5 and 0.4 mols/L.

The total phenolic content was determined by the method of Singleton and Rossi (1965), which uses the reagent Folin–Ciocalteu and catechin as standard. The results were expressed as catechin equivalents. To assess the antioxidant capacity of the extract, the DDPH methods were used, according to Nishiyama et al (2010) and ABTS, according to Carvajal et al. (2012).

Preparation of microcapsules

The microcapsules were prepared by two methodologies, one using maltodextrin as an encapsulating matrix and the other using beta-CD. To obtain the microcapsules with maltodextrin, 10 g of the matrix was dissolved in 50 mL of distilled water. The pH was adjusted to 2.0 with 2 mol/L HCl and then 1 g of the lyophilized juçara extract was added to the solution. The mixture was stirred for 15 min on a magnetic stirrer, frozen, and lyophilized at – 50 °C for 36 h (Selim et al. 2008).

To obtain the microcapsules with beta-CD, 4 g of the matrix was “kneaded” with a pistil and a grail with 4 mL of distilled water, at room temperature, for 5 min. Subsequently, 0.4 g of lyophilized juçara extract was added and kneading followed for another 20 min. The mixture was frozen and lyophilized at – 50 °C for 36 h (Marcolino et al. 2011).

The efficiency of the microencapsulation process

The efficiency of the microencapsulation process was measured according to the methodology used by Passos et al (2015), which analyzes the content of anthocyanins present inside and outside the formed microcapsule. To measure anthocyanins inside the microcapsules, 200 mg of the microcapsules were weighed, and 2 mL of the methanol: acetic acid: water solution was added at a ratio of 50:8:42 (v/v/v). The mixture was stirred for 1 min and homogenized in heating ultrasound at 37 °C, in two times of 20 min. Subsequently, the anthocyanin content was measured according to the differential pH method already mentioned.

For the determination of anthocyanins outside the microcapsules, 200 mg of the microcapsules were weighed and 2 mL of ethanol: methanol solution was added in a 1:1 (v/v) ratio. The mixture was stirred for 1 min and filtered through a Millipore filter (45 µm, 13 mm) (Millipore Corporation, Swinney Stainless, Bedford, MA 01,730, USA). The anthocyanin content was measured according to the differential pH method already mentioned. The percentage of surface compounds (CS) and the efficiency of the microencapsulation process (EM) were calculated according to Eqs. (1) and (2), respectively.

$$\text{CS (\%)} = \frac{\text{Surface compounds}}{\text{Theoretical total of compounds}} \times 100 \quad (1)$$

$$\text{EM (\%)} = 100 - \text{CS} \quad (2)$$

Characterization of microcapsules by FTIR-ATR, DSC, and TGA

FTIR-ATR spectra were obtained from lyophilized juçara extract, beta-CD, maltodextrin, microcapsules of the extract with maltodextrin and beta-CD, and a simple physical mixture of the extract with maltodextrin and beta-CD using an infrared Fourier transform spectrometer (model Vertex 70v, Bruker, Germany). The measurements were made in a range of 400 to 4000 cm⁻¹, with 128 scans and a resolution of 4 cm⁻¹.

The same samples were placed in platinum capsules and analyzed simultaneously by the Differential Scanning Calorimetry (DSC) and Thermogravimetry (TGA) techniques. The equipment (STA 409PG Luxx/NETZSCH, Selb, Germany) was operated at room temperature up to 600 °C, with a heating rate of 10° C/min and in a nitrogen atmosphere (20 mL/min).

Study of microcapsules stability to light and pH

The stability of juçara extract and microcapsules under light was analyzed according to the proposal by Mangolim et al

(2014), with modifications. The samples (2 g) were stored in two polyethylene embain packages with an area of 100 cm². One package of each sample was exposed to four 40 W fluorescent lamps as sources of artificial light, arranged perpendicularly and suspended at 2.10 m from the samples. The samples were stored for 40 days at room temperature and the content of anthocyanins was measured every 4 days according to the differential pH method already mentioned.

The stability of the extract and microcapsules against pH was evaluated according to the methodology of Wang et al (2010), with modifications. The samples were (5 mg) added to buffers with pH values ranging from 1.5 to 9 in test tubes protected from light. After homogenization, the levels of anthocyanins were read according to the differential pH method already mentioned.

Fermented milk application

Preparation of fermented milk

The lyophilized juçara extract and the microcapsule of the extract with maltodextrin were used in the preparation of two formulations of fermented milk, one with the addition of pure extract and the other with the addition of the microcapsule with maltodextrin.

The fermented milk was prepared by the addition of a milk culture of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* (Bio Rich®, Christian Hansen) in 2 L of UHT whole milk at 42 °C, which was kept in an oven for 6 h at this same temperature. Subsequently, the product was cooled to 4 °C and 10% (w/v) of refined sugar and 0.1% of the extract or microcapsule were added (Rensis and Souza 2008).

Color analysis

Color analysis was performed using a Minolta colorimeter (Konica Minolta, model CR 400, China), with reflectance reading of the coordinates L* (luminosity), a* (intensity of + red and –green), and b* (intensity of + yellow and –blue). The values for each sample were obtained using an average of three readings. The color was measured every four days, during 24 days of storage at 4 °C for the two fermented milk.

Statistical analysis

The results obtained were evaluated using analysis of variance (ANOVA) and Tukey's post-test ($p < 0.05$) for comparison between the samples evaluated, using Sisvar version 5.7 (Build 91). All tests were performed in triplicate and the results were described as mean ± SD (standard deviation).

Results and discussion

Total anthocyanins, phenolic compounds, and antioxidant activity of the extract

Table 1 presents the results of total anthocyanin content, total phenolic compounds (in catechin equivalents), and antioxidant activity (by the methods of DPPH and ABTS) of the juçara extract obtained from the pulp.

The content of total anthocyanins in the extract (Table 1) was quite high when compared to the pulp of fresh fruit, which, according to Costa et al (2012), has anthocyanin content equal to 110.09 mg/100 g. When compared to the dehydrated pulp by spraying, which presented 7079 ± 83 mg/100 g of anthocyanins in the study by Pereira et al (2020), it is still clear that the extract concentrated these compounds, as they present 52% more anthocyanins than the dehydrated pulp. In addition to the concentration of coloring compounds, the use of juçara extract by the food industry as a substitute for fresh or dehydrated pulp shows an advantage for being highly soluble in water and free of lipophilic interferences, which favors its application as a natural dye in product development.

Also, according to Table 1, the lyophilized juçara extract had a higher content of phenolic compounds than those found by Garcia et al (2019), who studied the composition of phenolic compounds for the hydroethanolic bark extract (1320 mg/100 g) and by Inada et al (2015) who evaluated the content in the juçara pulp (1780 mg/100 g of dry pulp weight). This superiority is due to the concentration of anthocyanins in the extract since the main constituents of the phenolic compounds of juçara are anthocyanins, especially cyanidin-3-rutinoside and cyanidin-3-glycoside (Schulz et al. 2016).

The anthocyanins and the other phenolic compounds in the extract gave it significant antioxidant activity, both by the DPPH radical method and by the ABTS radical method (Table 1). Similar results were described by Garcia et al. (2019) for the extract of juçara bark. The authors observed significant antioxidant activity both for the capture of ABTS radicals (23.2 ± 0.3 TEAC $\mu\text{mol TE/mg}$) and for the DPPH radical (13.1 ± 0.2 TEAC $\mu\text{mol TE/mg}$).

Table 1 Content of anthocyanins, total phenolic compounds and antioxidant activity (EC_{50}) evaluated by the methods of DPPH and ABTS for the lyophilized extract rich in anthocyanins obtained from juçara pulp

Anthocyanin content (mg/100 g)	Total phenolic compounds (mg/100 g)	EC_{50} DPPH method ($\mu\text{g/mL}$)	EC_{50} ABTS method ($\mu\text{g/mL}$)
$10,780 \pm 765$	$12,900 \pm 300$	33 ± 05	10.12 ± 0.24

The literature describes that, depending on the IC_{50} value, plant extracts can be classified as highly active ($IC_{50} < 50$ $\mu\text{g/mL}$), moderately active ($50 < IC_{50} < 100$ $\mu\text{g/mL}$), weakly active ($100 < IC_{50} < 200$ $\mu\text{g/mL}$) or inactive ($IC_{50} > 200$ $\mu\text{g/mL}$) (Rai et al. 2017). Therefore, from the observed results, it is possible to verify that the extract obtained is highly active. This antioxidant potential is very interesting in the development of functional food products, since compounds with this bioactivity can act in health promotion (Tarone et al. 2020).

The efficiency of microencapsulation, characterization, and stability of microcapsules

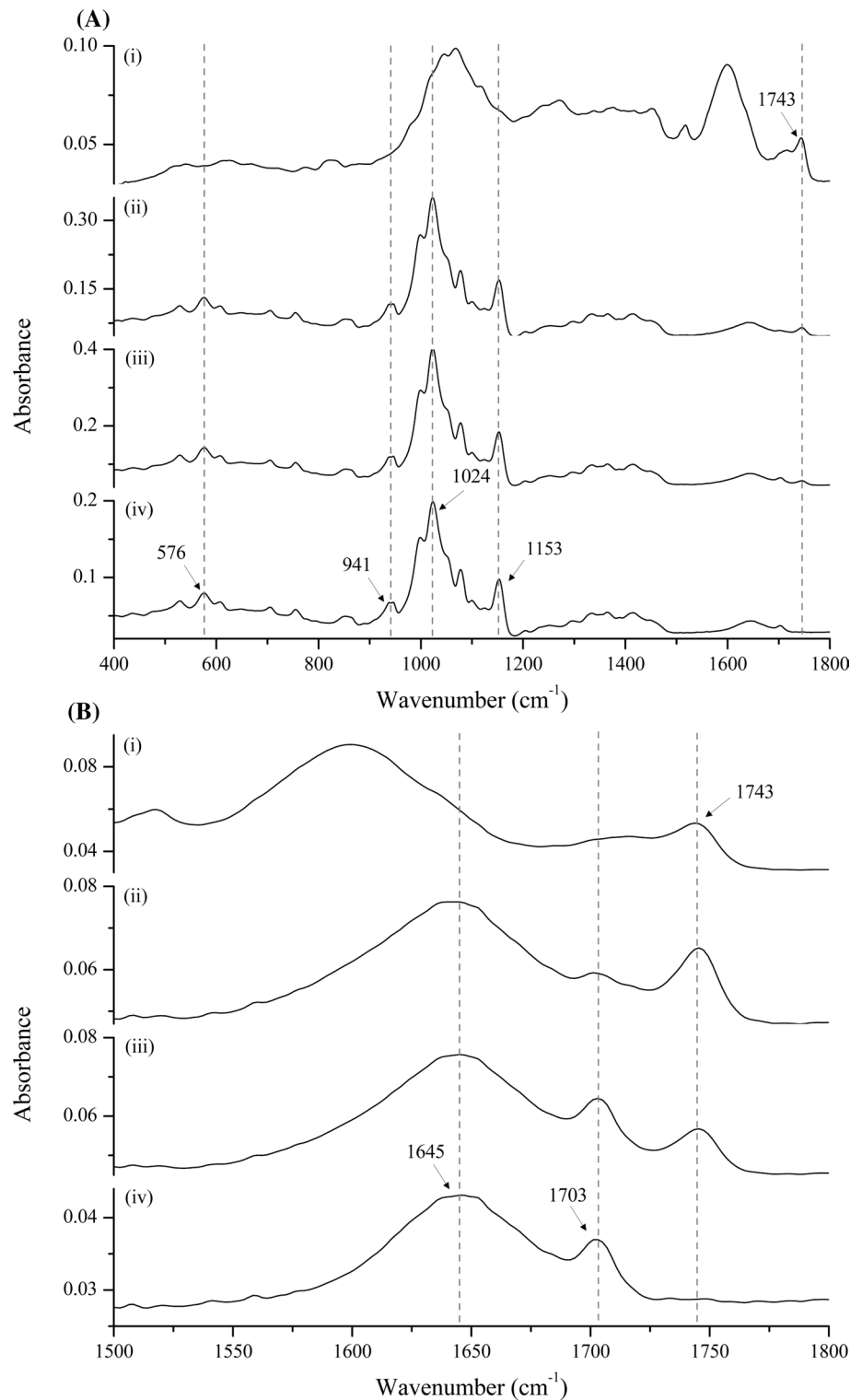
The microencapsulation of the anthocyanin-rich extract with the maltodextrin and beta-cyclodextrin matrices showed an efficiency of 89.1 and 80.9%, respectively. The greater affinity of the extract for maltodextrin is due to the hydrophilic character of the extract and the matrix, while beta-cyclodextrin has a greater capacity to encapsulate lipophilic compounds (Mangolim et al. 2014).

Superior encapsulation efficiency results were achieved by Passos et al (2015), who achieved 93.6% efficiency when encapsulating juçara anthocyanins with maltodextrin by lyophilization. Mazuco et al (2018) obtained lower results when encapsulating juçara pulp with maltodextrin and gum arabic by freeze-drying and atomization, reaching values between 64.06 and 83.69%. The efficiency of the microencapsulation process can be influenced by different parameters, such as the properties of the encapsulant, the size and physical–chemical parameters of the particles, the encapsulation technique employed, among others.

The characterization of the obtained microcapsules was carried out by the FTIR-ATR technique, and by the thermal analyzes of DSC and TGA. Figure 1 illustrates the FTIR-ATR result of the samples of pure juçara extract, pure beta-CD, simple mixture between the extract and beta-CD, and the extract-beta-CD microcapsule.

In Fig. 1A, it is possible to observe that the spectra of the sample mixture and the microcapsule show great similarity with the spectrum of beta-CD, with emphasis on the peaks at 576, 941, 1024, and 1153 cm^{-1} . Among these, the most characteristic peaks of beta-CD are in the region between 1000 and 1180 cm^{-1} and can be attributed to the stretching of the C–O–C and C–OH bonds of the molecule, with the main peak in 1024 cm^{-1} (Banjare et al. 2020). Still, in Fig. 1A, it can be seen that the characteristic peak of the extract that appears in the spectra of the sample mixture and the microcapsule, even at low intensity, is 1743 cm^{-1} , being attributed to the carbonyl stretch C=O (Westfall et al. 2020), located in the central region of cyanidins (3-glycoside and 3-rutinoside), which are the main anthocyanins of juçara.

Fig. 1 FTIR-ATR spectra of (i) Juçara extract, (ii) Juçara extract microcapsule with beta-CD (iii) Simple mixture of juçara extract with beta-CD and (iv) Beta-CD (A); zoom in the region $1500\text{--}1800\text{ cm}^{-1}$ of Fig. 1A of (i) Juçara extract, (ii) Microcapsule of juçara extract with beta-CD (iii) Simple mixture of juçara extract with beta-CD and (iv) Beta-CD (B). The dotted lines reveal characteristic peaks in the samples or peaks that have undergone modifications



Despite the great similarity between the spectra of the mixture and the microcapsule, in the zoom of the $1500\text{--}1800\text{ cm}^{-1}$ region (Fig. 1B) there is a differentiation. Both samples showed peaks in 1703 cm^{-1} (characteristic of beta-CD) and 1743 cm^{-1} (characteristic of the extract). However, the intensities are reversed, because for

the microcapsule the ratio between the intensities of these peaks (I_{1743}/I_{1703}) is equal to 1.10 and, for the mixture, I_{1743}/I_{1703} is equal to 0.88, which may indicate a molecular interaction between the anthocyanins from the extract and the beta-CD molecule. However, this interaction does not characterize molecular inclusion, since the peaks of

the beta-CD involved are attributed to the hydration water of the molecule (the peak at 1645 cm^{-1} which is joined to the peak at 1703 cm^{-1} refers to H–O–H), while other studies involving molecular inclusion in cyclodextrins (Mangolim et al. 2014; Banjare et al. 2020) report changes in peaks in the region between 1000 and 1180 cm^{-1} , which are attributed to stretches of bonds located in its hydrophobic cavity.

Figure 2 illustrates the FTIR-ATR result of the samples of pure juçara extract, pure maltodextrin, the simple mixture between the extract and maltodextrin, and the extract-maltodextrin microcapsule.

Figure 2 reveals that the spectra of the extract-maltodextrin microcapsule and the simple mixture present the same peaks of the maltodextrin spectrum, with emphasis on the peaks at 1016 , 1078 , and 1149 cm^{-1} . According to Maqsoudlou et al (2020), the peaks in the region between 850 and 1170 cm^{-1} are related to vibrations of anhydrous glucose stretch (more especially to the C–O stretch), since, in the binding of glucose molecules in the structure of maltodextrin, a molecule of water is released and creates the structure of anhydroglucose. Still, in Fig. 2, the only peak of the extract that appears in the mixture and the microcapsule is 1743 cm^{-1} , already revealed to be attributed to the carbonyl stretch C=O (Westfall et al. 2020) of the anthocyanin molecules. The spectrum of the microcapsule, due to the low intensity of the absorbance, presents a lot of noise, but the similarity between the spectrum of the microcapsule and the mixture is clear, showing no molecular interaction

between the extract and the maltodextrin in the formation of the microcapsule.

Figures 3A and 3B show the TGA and DSC curves for samples of pure juçara extract, pure beta-CD, the simple mixture between the extract and beta-CD, and microcapsule extract-beta-CD. Figures 3C and 3D show the TGA and DSC curves for samples of pure juçara extract, pure maltodextrin, the simple mixture between the extract and maltodextrin, and microcapsule extract-maltodextrin.

Figures 3A and 3B show that there was no molecular inclusion or microencapsulation between the beta-CD and the extract, as both the simple mixture and the microcapsule revealed loss of mass (Fig. 3A) and DSC peaks (Fig. 3B) similar to pure cyclodextrin. DSC is widely used to confirm molecular inclusion in a solid-state and, according to Mehran et al (2020), the lack of additional peaks in the microcapsule DSC compared to the matrix suggests that there was no interaction between the matrix and the extract. Different data were found by Fernandes et al (2018), who noticed DSC peak displacements when microencapsulating the blackberry cyanide-3-glycoside molecule with beta-cyclodextrin, proving the formation of the inclusion complex between the involved molecules.

Even so, beta-CD promoted heat stability to the extract. Beta-CD showed a peak of water loss at 130 °C , losing 12% of water mass up to that temperature. This first loss of mass at a temperature below 200 °C corresponds to the evaporation and desorption of water due to the hydrophilic nature of the encapsulant (Villacrez et al. 2014). Subsequently, its

Fig. 2 FTIR-ATR spectra of (i) Juçara extract, (ii) Microcapsule of juçara extract with maltodextrin (iii) Simple mixture of juçara extract with maltodextrin and (iv) Maltodextrin

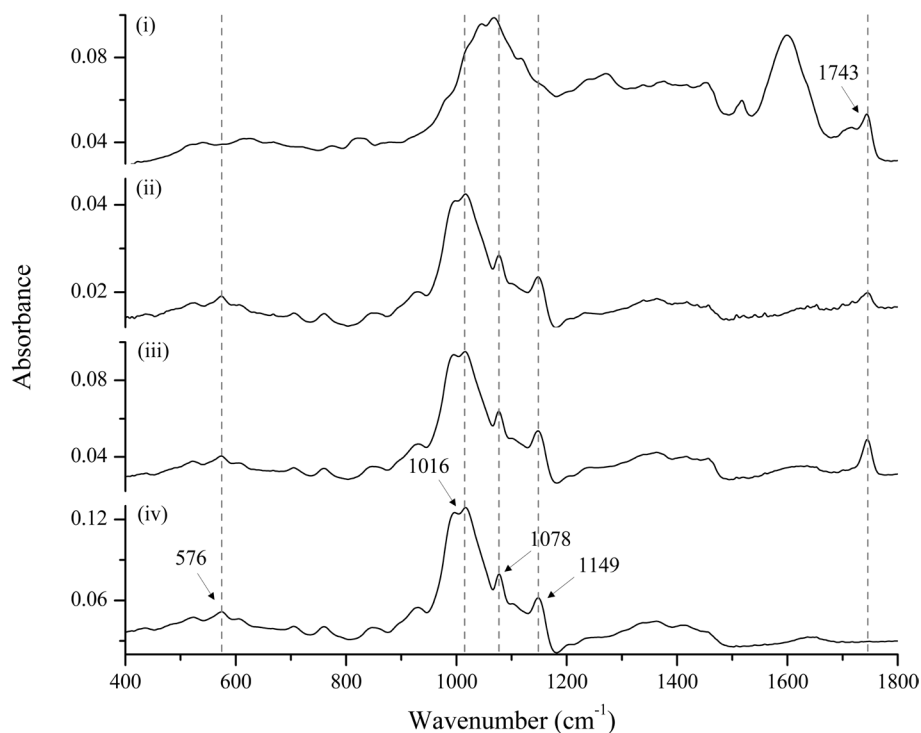
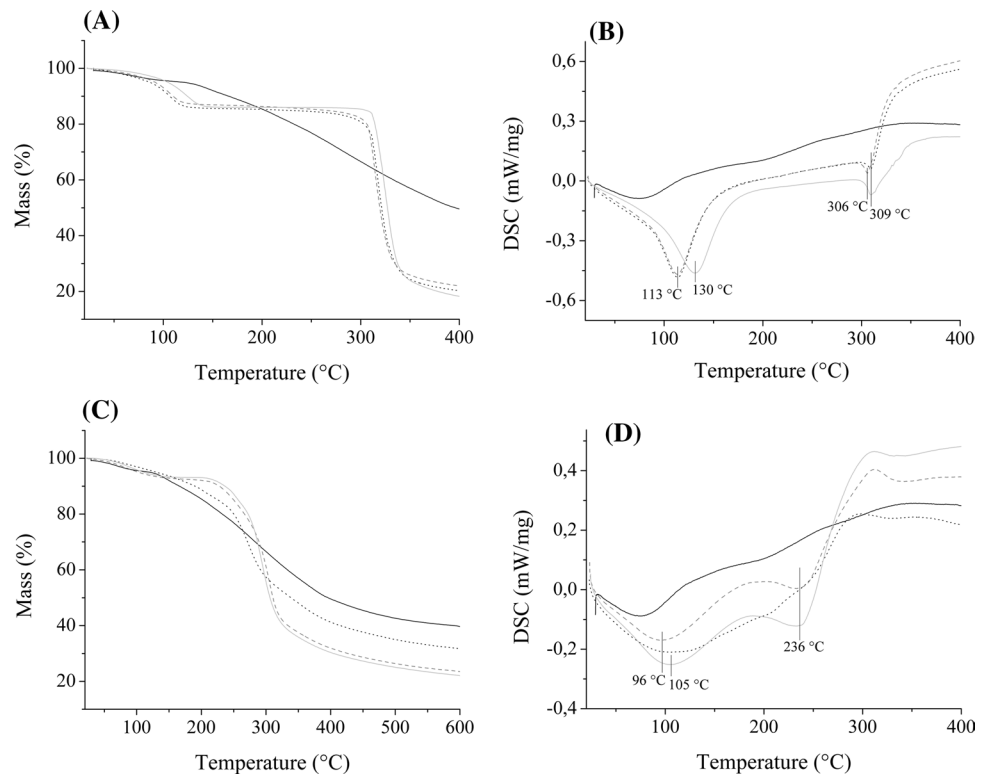


Fig. 3 TGA (A) and DSC (B) curves of samples of juçara extract (solid black line), beta-CD (continuous gray line), simple mixture between extract and beta-CD (dashed gray line) and microcapsule extract–beta-CD (black dotted line); TGA (C) and DSC (D) curves of samples of juçara extract (solid black line), maltodextrin (continuous gray line), simple mixture between extract and maltodextrin (dashed gray line) and extract–maltodextrin microcapsule (dotted line) (black)



mass remained practically stable until its degradation, which occurred abruptly at 309 °C. The microcapsule and simple mixture samples showed the same behavior, as they also lost 12% of mass up to the water loss temperature (which occurred at 113 °C) and, up to their degradation temperature, which was 309 °C for the microcapsule and at 306 °C for the simple mixture, they presented additional weight loss of 7.5 and 11%, respectively. The peak of degradation of the pure extract disappeared in the DSC of the microcapsule and the simple mixture, proving the thermal protection conferred.

The peak of degradation of the extract was very subtle and broad, in the region of 200 °C, a data that corroborates with that found by Mehran et al (2020), who identified the peak of anthocyanins from Iranian borage petals in 215 °C. The pure extract showed high instability to heating, as it slowly lost mass up to 120 °C (until it lost only 5% of its mass) and, after that temperature, it lost mass constantly, with an approximate rate of 0.2% mass/°C. The increase in temperature causes loss of glycosides in anthocyanins, which results in aglycones that are more unstable and cause the accelerated degradation of molecules (Mehran et al. 2020).

In Figs. 3C and 3D, a different behavior was observed, as there were shifts in the microcapsule thermogram of the maltodextrin–extract compared to both the pure extract thermogram and the thermograms of the simple mixture and of the pure maltodextrin, which, according to Mansour et al (2020), is evidence of true encapsulation. The microcapsule showed moderately constant loss of mass up to 236 °C (the

temperature at which there was no defined peak), reaching a 17% loss of mass at this temperature. The simple mixture showed a peak of water loss at 96 °C, with a loss of mass of 4.5%, and a melting peak at 236 °C, with a loss of total mass of 12%. The same happened with maltodextrin, which presented the peaks mentioned at 105 and 236 °C and a 10% loss in mass at the last temperature. The melting temperature of maltodextrin is consistent with that found by Mazuco et al (2018), who found the melting point of the molecule between 240 and 250 °C.

Figures 3C and 3D also revealed that maltodextrin promoted moderate thermal stability to the extract, this stability is inferior to that found by the simple mixture between maltodextrin and the same. Microencapsulation caused a change in the mass loss observed in Fig. 3C, but the amplitude of the peaks in Fig. 3D made it difficult to identify the anthocyanins degradation temperature in the microcapsule. The results obtained revealed that the microencapsulation promoted physical interactions of the molecule with the extract by modifying its thermal properties when compared to the simple mixture, but that the thermal stability, despite having been improved with the pure extract, was not as significant as only in a simple mixture between the substances involved.

Other authors obtained both evidence of microencapsulation and improved thermal stability of anthocyanins encapsulated with maltodextrin. Mehran et al (2020), when encapsulating anthocyanins from Iranian borage petals with

maltodextrin and modified starch realized that the peak of anthocyanin degradation disappeared in the microcapsule's DSC thermogram, indicating that the anthocyanins were completely protected by the matrix. Villacrez et al (2014), when microencapsulating anthocyanins from Andean raspberry with maltodextrin, perceived moderate thermal stability in the microcapsule, with significant loss of mass above 200 °C, which is attributed to the degradation of the polysaccharide.

Figure 4 shows the light and pH stability of the pure and microencapsulated extract with maltodextrin and beta-cyclodextrin.

As seen in Fig. 4A, the juçara extract showed a great drop in the anthocyanin content in the interval from 0 to 4 days of exposure to light, reaching 66% of dye retention. After this day, the drop in dye retention was greatly reduced, approaching stability. At the end of 40 days of exposure, the extract showed 56% retention of anthocyanins. The microcapsules with maltodextrin and beta-CD showed better stability with exposure to light, with a more significant drop in dye retention between days 36 and 40. In the end, these samples showed 80 and 86% retention of anthocyanins (microcapsule beta-CD and maltodextrin, respectively). Authors such

as Lacerda et al (2016) also found that some encapsulating carbohydrates protected the juçara pulp from degradation conditions. The authors encapsulated the pulp with modified starch, inulin, and maltodextrin (separated and combined) and noticed an improvement in stability under artificial light, heating at 50 °C and storage for 38 days.

Figure 4B reveals that, to pH variation, the behavior of extract microcapsules with beta-CD and with maltodextrin in solution was very similar to the extract up to pH 6. However, from pH 7, both microcapsules showed an increased absorbance when compared to pure extract, which reveals an increase in the stability of these compounds in alkaline pH. According to Passos et al (2015), anthocyanins prevail red in acidic pH, colorless in pH close to neutrality, and with unstable coloring in alkaline pH. These same authors found very similar absorbance values when comparing pure juçara extract with microcapsule with maltodextrin at pH values 6 to 9. These values were very different to those found in the present study, in which at pH 7, 8 and 9 increased absorbance (or color intensification) was 57, 77, and 86%, respectively, for the microcapsule with maltodextrin and 81, 104, and 118%, respectively, for the microcapsule with maltodextrin.

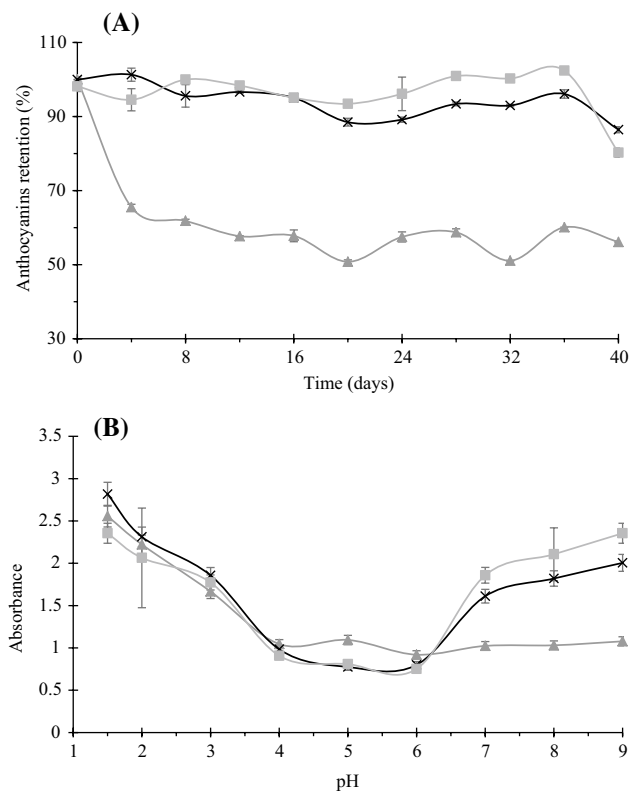


Fig. 4 Stability of the anthocyanin-rich extract on exposure to light for 40 days (A) and stability against different pH values (B). Square = microcapsule with beta-CD; x = microcapsule with maltodextrin; triangle = pure extract

Fermented milk application

The microcapsule with maltodextrin was chosen for application in fermented milk. The choice was made due to the lower cost of the encapsulant, the fact that it presented better encapsulation efficiency, better light stability, good pH stability, and the microcapsule formation was evident in the analysis of DSC and TGA. Table 2 shows the colorimetric coordinates of the fermented milk with pure extract and microencapsulated with maltodextrin during the storage of the products for 28 days.

Table 2 reveals that, to the luminosity parameter (L^*), the fermented milk with pure extract and microencapsulated with maltodextrin showed similar values and the same light browning behavior with storage, as both increased the parameter by 4% during the 24 days. The results of parameter a^* , which refer to the red color of the sample, showed that up to 12 days of evaluation there was no difference between the samples, however, after 16 days, it was possible to identify that the use of microencapsulated material provided greater product stability in red color, as the parameter reduction was 60% for milk fermented with pure extract and 35% for milk fermented with the microencapsulated extract.

Similar behavior was observed for the yellow color of the samples, that is, for the parameter b^* . Although the two formulations showed a gradual increase in yellowish color with storage, after 16 days they showed a significant difference, which also showed that the microencapsulation process

Table 2 Means ± standard deviation of the colorimetric attributes (L*, a* and b*) of fermented milk prepared with the addition of pure extract and with the addition of a microcapsule of the extract with maltodextrin in 24 days of storage

Day	L*		a*		b*	
	Extract	Microcapsule	Extract	Microcapsule	Extract	Microcapsule
0	67.36 ± 0.02 ^{Ab}	68.18 ± 0.01 ^{Ab}	10.57 ± 0.01 ^{Aa}	10.06 ± 0.00 ^{Aa}	5.16 ± 0.01 ^{ABc}	5.03 ± 0.01 ^{Bc}
4	69.2 ± 0.60 ^{Aab}	70.00 ± 1.00 ^{Aab}	8.70 ± 0.20 ^{Ab}	8.70 ± 0.60 ^{Ab}	7.2 ± 0.20 ^{Ab}	7.45 ± 0.08 ^{Ab}
8	69.00 ± 1.00 ^{Aab}	69.9 ± 0.72 ^{Aab}	7.90 ± 0.20 ^{Ab}	7.60 ± 0.20 ^{Abc}	7.6 ± 0.70 ^{Ab}	8.1 ± 0.20 ^{Aab}
12	69.00 ± 1.00 ^{Aab}	71.2 ± 0.10 ^{Aa}	6.00 ± 0.60 ^{Ac}	6.66 ± 0.09 ^{Ac}	7.9 ± 0.90 ^{Ab}	7.67 ± 0.01 ^{Aab}
16	69.00 ± 1.02 ^{Bab}	71.5 ± 0.10 ^{Aa}	4.90 ± 0.60 ^{Bcd}	6.70 ± 0.03 ^{Ac}	9.62 ± 0.01 ^{Aa}	7.19 ± 0.06 ^{Bb}
20	71.00 ± 0.80 ^{Aa}	71.1 ± 0.42 ^{Aa}	4.90 ± 0.80 ^{Bd}	6.70 ± 0.10 ^{Ac}	9.7 ± 0.10 ^{Aa}	8.7 ± 0.20 ^{ABab}
24	71.4 ± 0.10 ^{Aa}	72.13 ± 0.04 ^{Aa}	4.20 ± 0.80 ^{Bd}	6.50 ± 0.20 ^{Ac}	10.63 ± 0.03 ^{Aa}	9.2 ± 0.50 ^{Ba}

A, B, C Averages within the same line with capital letters denote a significant difference ($p < 0.05$) for each color parameter evaluated in each yogurt sample

a, b, c, d Averages within the same column with lowercase letters show a significant difference ($p < 0.05$), for the same color parameter evaluated over the days

provided greater stability in the coloring of anthocyanins from juçara extract applied to the food.

Similar results were described by Passos et al (2015) who also evaluated the color parameters L*, a*, and b* for yogurts with the addition of juçara microcapsules with maltodextrin. The authors showed that microencapsulation provided greater stability of the red color, especially when compared to food without microencapsulated extract. Also, Lima et al (2019) when preparing fermented milk drinks or not with the addition of microencapsulated juçara extracts with maltodextrin, realized that the microencapsulation contributed to the maintenance of the color of the drinks, both in opaque and transparent packaging, during the 28 days of storage.

Sensory analysis of the two types of fermented milk was performed (Supplementary material). The sensory preference for fermented milk with a microcapsule in all the attributes involved, except for the color in which the products have the same acceptance, suggests that the application of the microcapsule positively implies in the adopted sensory attributes. This preference can be explained by the benefits of including maltodextrin in the product, as a function of this ingredient goes beyond the encapsulating matrix, and can be used as a thickener and binder, often associated with the occurrence it produces in the mouth because it is a body agent in the food.

Conclusions

The use of beta-CD and maltodextrin as encapsulating agents of juçara extract showed significant thermal stability and in the light of anthocyanins, in addition to intensifying their colors in a broader pH range. However, the FTIR-ATR techniques and the thermal analyzes of DSC and TG showed that there was no molecular inclusion between the extract and beta-CD, but there was a physical

interaction between the maltodextrin and the extract, due to the hydrophilic characteristics of the materials involved. When applied in fermented milk, the microcapsule of the extract with maltodextrin showed better color stability of the product compared to the pure extract. Therefore, this research can contribute to the development of innovative food products, rich in anthocyanins, more stable, and with the possibility of good commercialization.

Additional electronic material in support of your Manuscript

This article has supplementary material. The additional material refers to the sensory analysis performed for fermented milk and this study was approved by the Standing Committee on Ethics in Research Involving Human Beings of Maringá State University (Protocol CAEE no. 45414821.3.0000.0104).

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Authors' contributions Thamara Thaiane da Silva Crozatti participated in the research planning, experimental analysis, interpretation of results and writing of the article. Camila Sampaio Mangolim participated in the research planning, execution and analysis of data and writing of the article. Paula Vitória Larentis collaborated in carrying out the laboratory analyses. João Carlos Palazzo de Mello acted in the planning and writing of the article, and Graciete Matioli, the work's supervisor, participated in the research planning, interpretation of results and correction of the final writing of the article.

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Data Availability Datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request. The authors declare full data transparency.

Code availability Not Applicable.

Declarations

Conflict of Interest The authors declare that they do not have any conflict of interest.

Ethical approval This study was approved by the Standing Committee on Ethics in Research Involving Human Beings of Maringá State University (Protocol CAAE no. 45414821.3.0000.0104).

Informed Consent Written informed consent was obtained from all study participants.

Consent for publication The authors declare that they have read and approved the manuscript and the authors are pleased to submit the manuscript to the *Journal of Food Science and Technology*.

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