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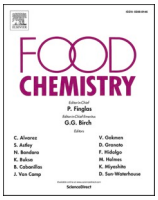

Development of alginate/pectin microcapsules by a dual process combining emulsification and ultrasonic gelation for encapsulation and controlled release of anthocyanins from grapes (*Vitis labrusca* L.)

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ABSTRACT

The aim of this study was to investigate the physicochemical, morphological, and gastrointestinal release properties of an anthocyanin-rich extract of grapes in alginate and pectin beads as carriers; the effects of ultrasonic gelation combined with emulsification were also investigated. In general, the alginate beads showed smaller size and more regular shape compared to pectin. The effect of emulsification combined with ionic gelation was more pronounced in the alginate beads and resulted in higher retention of anthocyanins, higher antioxidant capacity, and also allowed the best release profile during intestinal digestion. Thus, the simultaneous strategy could be an interesting delivery system and enhance the release of anthocyanins, providing an opportunity for the development of ingredients with different bioactive properties.

1. Introduction

In recent decades, consumer demand for antioxidants from natural sources has increased in importance due to the trend toward healthier and more balanced diets. They are seeking out additional benefits such as health promotion and disease prevention. On this feel, grapes occupy an outstanding location as a supply of phenolic compounds associated with the advertising of human health, acting, for instance, as a natural antioxidant and minimizing the outcomes of premature aging (Asioli et al., 2017).

Grape production occupies a prominent position in world cultivation and is considered an important fruit for the agro-industrial sector. It is consumed both in fresh form, *in natura*, and for the preparation of various foods such as jellies, kinds of vinegar, oils, juices, and wines. However, tons of agro-industrial by-products such as seeds, stems, and hulls are generated during processing. It is estimated that only 3% of these byproducts are reused, as they are considered low value-added wastes and are often used as soil fertilizer, to supplement animal feed, or even incinerated. Therefore, it is mandatory that there is economic and environmental responsibility for a more appropriate destination for these byproducts (Dávila et al., 2017).

Grape by-products, especially skins, represent a promising and cost-effective source of natural phenolic compounds for the food industries. They are responsible for biological activities such as: antioxidant, antidiabetic, antimutagenic, anti-inflammatory and prevention of coronary diseases (Dávila et al., 2017). Among these interesting phenolic compounds, anthocyanins stand out.

Anthocyanins are hydrophilic non-toxic natural pigments and are found in flowers, vegetables and fruits. Several studies have shown the biological effects related to anthocyanins, such as: antioxidant and anti-inflammatory properties, suggesting that they may have the potential to prevent obesity, colon cancer and diabetes. Thereby, it is suggested that the ingestion of anthocyanins could be attributed to the improvement of several parameters related to the intestinal health (Han et al., 2021). However, they have low stability under unfavorable conditions and are affected by the environment (temperature, pH, oxygen and light), interaction with other components and gastrointestinal conditions (pH and enzymes) (de Moura, Berling, Germer, Alvim, & Hubinger, 2018). Therefore, the main challenge is to find technologies to preserve and incorporate these phenolic compounds into food products. These

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limitations can be overcome by microencapsulation.

Among the various microencapsulation techniques used for the protection of phenolic compounds, ionic gelation is very promising. It is considered a simple technique that requires mild preparation conditions and does not require temperature or toxic solvents to the environment (McClements, 2017). Various atomization nozzles are available for ionic gelation and the selection must take into account relevant factors such as the desired size and size distribution of the microparticles and also their final application. Recently, ultrasonic nozzle has emerged as an interesting alternative for the preparation of microparticles with smaller, uniform and spherical size, facilitating their dispersion in various products, such as foods, and also leads to better sensory acceptance by consumers due to the properties of the microparticles obtained by ultrasonic gelation (Barba, Dalmoro, d'Amore, & Lamberti, 2015; Turan et al., 2016). The anionic polymers sodium alginate and low amidated pectin are the most commonly polymers used on the development of microparticles by ionic gelation (McClements, 2017).

Alginates are nature-based water-soluble polysaccharides found in some bacteria and brown algae. Their chemical structure consists of residues of a linear 1,4-linked copolymer of L-guluronic and D-mannuronic acids. It is biodegradable and biocompatible, and the interaction of Ca^{2+} are responsible for the 3D-network gel formation (Comunian & Favaro-Trindade, 2016; Hambleton, Debeaufort, Bonnotte, & Voilley, 2009; McClements, 2017; Zia, Zia, Zuber, Rehman, & Ahmad, 2015).

On the other hand, pectin consists of a polysaccharide fiber with a very complex structure. Its chemical structure is formed by linear units of galacturonic acid linked by partially methoxylated glycosidic bonds. Its outstanding properties include biocompatibility, biodegradability and gelling capacity. In addition, pectins are consider safe for consumption (GRAS) and low cost (Wicker & Kim, 2015).

However, the hydrogel beads produced with these polymers are porous, which may compromise the release and protection of the microencapsulated hydrophilic anthocyanins. An alternative to overcome this technical obstacle is to combine microencapsulation and emulsion systems. When dispersed in emulsion gels, bioactive ingredients are protected and immobilized by the interaction between the emulsion and the network formed, and therefore show an improvement in physicochemical properties from adverse conditions, control their release, and increase their release during digestion (Beldarrain-Iznaga, Villalobos-Carvajal, Leiva-Vega, & Sevillano Armesto, 2020; Geremias-Andrade, Souki, Moraes, & Pinho, 2016). Recently, the efficacy of gel microparticles for encapsulating bioactive compounds has been showed several times (Brito-Oliveira et al., 2017; Chen et al., 2018; Feng et al., 2018; Geremias-Andrade et al., 2016; Hou, Guo, Wang, & Yang, 2016; Mokhtari, Jafari, & Assadpour, 2017; Mun, Park, Kim, & McClements, 2016; Nayak, Hasnain, Beg, & Alam, 2010; Torres, Tena, Murray, & Sarkar, 2017).

In the present work, the potential use of microencapsulation by ion gelation in combination with an emulsification process was investigated to improve the stability of hydrophilic natural anthocyanins extracted from the grape skin. It is worth noting that this is the first study in which a combined approach of emulsification and ionic ultrasonic gelation with alginate/pectin as gelling agent was used. The microcapsules were evaluated for their moisture content, water activity, mean size, morphological properties, anthocyanin content, total phenolic compounds, antioxidant capacity, encapsulation efficiency (EE), vibrational spectroscopy and gastrointestinal release. The results of this study could allow the development of active microparticles to meet the current market trends in the food sector and also provide an alternative for the preservation of the physicochemical properties of anthocyanins.

2. Materials and methods

2.1. Materials

The grapes of the Bordô variety (*Vitis labrusca* L.) were kindly donated by producers from a farm located in Campos Gerais (Minas Gerais, Brazil). Hydrogel beads were prepared using sodium alginate (Sigma-Aldrich, Darmstadt, Germany); low methoxyl amidated pectin LM-104 AS-Z Genu® (CP Kelco, Limeira, SP, Brazil); calcium chloride (Anidrol, Diadema, SP, Brazil); soybean oil (Bunge Alimentos, Brazil) and polyglycerol polyricinoleate (PGPR) emulsifier (Concepta Ingredients, SP, Brazil).

2.2. Preparation of the grape peel extract (GPE)

The grapes were washed and disinfected. To obtain the grape peel extract, the seeds, peel, and pulp were removed manually. Anthocyanins from the peel were extracted with distilled water and citric acid (1% wt. of water) in a 1:1 (w/v) ratio (peel: acidified water). The mixture was kept overnight in the dark at a refrigeration temperature of 5 ± 1 °C and covered with aluminum foil to improve the extraction of anthocyanins. Then, the extract was filtered through organza and centrifuged at 5,000 rpm to remove suspended solids. Finally, the grape peel extract (GPE) was stored in amber bottles to avoid degradation of anthocyanins at a refrigeration temperature of 5 ± 1 °C.

2.3. Encapsulation by ultrasonic ionic gelation method

For the preparation of control microspheres, gelation of alginate (ALG) and pectin (PEC) was performed according to the adapted method of Belščak-Cvitanovic et al. (2015). The alginate or pectin solutions (2%, w/w) were prepared by dissolving the polymer in the previously prepared grape skin extract (GPE) and homogenized for 20 min at 10,000 rpm in an Ultra-Turrax. The particles were atomizer on the cross-linking solution ($CaCl_2$ – 1.5% w/w) using an ultrasonic nozzle (0.5 mm diameter) and with a flow rate of 0.565 mL/min. The beads were stirred at 150 rpm for 30 min. Then, they were filtered with a vacuum pump, dried at 30 °C in a vacuum oven until completely dry, and stored in aluminum pouches.

The combined emulsification and ionic gelation for microencapsulation was performed following the methodology proposed by Moura et al. (2018). The primary w_1/o emulsion was prepared as follows. First, a measured amount of PGPR (4% w/w) was dissolved in soybean oil to prepare the oil phase. Then, grape skin extract was added at a ratio of 35:65 w/w and mixed for 15 min and 7,000 rpm in an Ultra-Turrax. The double emulsion ($w_1/O/w_2$) was prepared with the polysaccharide (pectin or alginate) solution (2% w/w) in an Ultra-Turrax IKA operating at 7,000 rpm for 10 min at a ratio of 20:80 w/w. A ultrasonifier (30 °C, 200 W) (Model S-450D, Branson Ultrasonic Corporation, Danbury, USA) was also used for emulsification for 7 min. Samples contain pectin (PEC - DE) and alginate (ALG - DE) were prepared according to the aforementioned gelation steps of the control microbeads.

2.4. Microparticles characterization

2.4.1. Moisture content and water activity

The moisture content of the dried samples was determined using the infrared radiation in a thermobalance (Moisture Balance MOC-120H; Shimadzu Corporation, Tokyo, Japan), in triplicate, at 70 °C until constant weight. The water activity (a_w) of samples (1 g of each) was measured in triplicate by using a hygrometer HygroPalm AW1 (Rotronic Instruments, Huntington, NY, USA) at 25 °C.

2.4.2. Mean diameter and size distribution

The beads were homogenized in isopropanol as dispersing medium using a sonifier (Model S-450D, Branson Ultrasonics Corporation, Danbury, USA) for 1 min at a power of 200 W. De Brouckere mean diameter ($d_{4,3}$) and diameter distribution (indicated by polydispersity index, PdI) were determined using a Mastersizer (Model 3000E, Malvern Instruments Inc., Worcestershire, U.K.).

2.4.3. Morphology

Particle morphology was observed using an optical inverted light microscope (Carl Zeiss Sports Optics, model Axio Scope.A1, Zeiss, Germany), with 40X magnification. The acquisition of the images was carried out through the software AxioVision Rel. 4.8.

2.4.4. Particles dissolution to bioactive quantifications

The samples with extract were placed in sodium citrate solution in order to promote the disorganization of the gel structure and release the phenolic compounds present in the beads. So, 1.5 g of wet particles were dissolved in sodium citrate 3% (w/v) and kept in an ultrasonic bath for 2 h. The samples were filtered, transferred into a flask covered with aluminum foil and the subsequent analysis were performed: anthocyanin content, content of total phenolic compounds and antioxidant assay.

2.4.5. Anthocyanin content

The pH differential method (AOAC, 2006) was used to estimate the total anthocyanin content. Aliquots of the samples were placed at pH 1 and 4.5 and incubated at room temperature for 15 min. Then, the absorbance was read using a UV–Vis- spectrophotometer against a blank at 520 and 700 nm.

2.4.6. Content of total phenolic compounds

About 1.5 ± 0.01 g of sample was used to determine the total phenolic compounds according to the Folin-Ciocalteu method (Turfan, Türkyilmaz, Yemi, & Özkan, 2011). The absorbance of the samples was made in a UV–Vis- spectrophotometer (model UV 2600i, Shimadzu, Kyoto, Japan) at 760 nm.

2.4.7. Antioxidant assay

About 1.5 ± 0.01 g of sample was used to evaluation of antioxidant activity by DPPH method performed according Brand-Williams, Cuvelier, & Berset (1995). The absorbance readings were made in a UV–Vis-spectrophotometer (model UV 2600i, Shimadzu, Kyoto, Japan). The results were expressed in mg of Trolox equivalent/g of dry beads.

2.4.8. Encapsulation efficiency (EE)

The encapsulation efficiency was calculated on dry basis according to De Moura et al. (2018) and was determined by Eq. (1).

$$\text{EE}\ (\%) = \frac{\text{mg of anthocyanin in microparticle}}{\text{mg of anthocyanin in the mixture}} \times 100 \quad (1)$$

For the control groups (ALG and PEC) the mixture was only composed of: grape peel extract + alginate/pectin solution. For ALG-DE and PEC-DE the mixture was composed of: emulsion (soybean oil + grape peel extract) + pectin or alginate solution.

2.5. FTIR vibrational spectroscopy

Fourier transform infrared spectroscopy (FTIR) of the microcapsules were obtained using a spectrophotometer (model Vertex 70v, Bruker, Massachusetts, United States). Measurements were done at 25 °C and with the recording from 4000 to 400 cm^{-1} at a scan rate of 40 scans with a 4 cm^{-1} spectral resolution.

2.6. Anthocyanin release profile under gastrointestinal conditions

The release profile of anthocyanins was carried out using an *in vitro* model according to Belscak-Cvitanovic et al. (2015). About 2.5 g of microencapsulated anthocyanins or free extract were dispersed in 50 mL of simulated gastric fluid (pH 1.2) and incubated at 37 °C in thermostatic bath with constant shaking for 120 min to mimic stomach conditions. Afterward, the microparticles were dispersed in 50 mL of simulated intestinal fluid (pH 7.4) and incubated at 37 °C for an additional 120 min. At defined time intervals (5–30 min), an aliquot of 5 mL of the fluids were taken for subsequent quantification of total monomeric anthocyanins (differential pH method).

2.7. Statistical analyses

For data analysis was applied a one-way analysis of variance (ANOVA), using the software OriginPro v9.9 (Origin Lab, Northampton, United States). The means differences were performed employing the Tukey test, with a significance level of α = 0.05%.

3. Results and discussion

3.1. Moisture content and water activity

Moisture content and water activity of grape powder beads are important parameters for predicting storage stability as they are related to microbial degradation rate and growth reactions (Geremias-Andrade et al., 2016). Table 1 shows the A_w and moisture content of dried pectin and alginate beads influenced by emulsification process.

According to the obtained results shown in Table 1, the moisture content of the beads varied between 2.9 and 9.1%. The higher moisture content was observed in the pectin-based beads when compared to the alginate ones. This tendency can be explained by the different packing density arrangement in the polysaccharides network, which affects the water affinity to the polymer components (Beldarrain-Iznaga, Villalobos-Carvajal, Leiva-Vega, & Sevillano Armesto, 2020; Sartori, Finch, Ralph, & Gilding, 1997).

ALG -DE and PEC- DE exhibited lower moisture content than ALG and PEC. This effect can be attributed to a less porous polymeric structure capable of adsorbing low-water content in their 3D-networks, because the oil (lipophilic) filling the space between the macromolecules (Belščak-Cvitanovic et al., 2016). Similar results were obtained by other authors who used alginate and pectin as gelling materials by ionic/ionotropic gelation (Beldarrain-Iznaga et al., 2020; Belščak-Cvitanovic et al., 2016). In relation to a_w, the values ranged from 0.37 to 0.43, values considered interesting since $a_w < 0.60$ can guarantee microbiological stability for food.

3.2. Mean diameter and size distribution

Particle size is a fundamental parameter in industrial applications of microencapsulation as it can be a limiting factor in food products (Belščak-Cvitanovic et al., 2016). The polydispersity index and mean droplet diameter of the beads generated by ultrasonic gelation technique are shown in Table 2.

All the beads showed a narrow distribution range, confirmed by the low PdI values, below 0.6, indicating a unimodal behavior with respect to size. ALG beads diameters were smaller than those of PEC. This difference could be attributed to the more reticulated structure of alginate, which promotes higher shrinkage of the polymer gel, resulting in lower diameters, when compared to pectin (Sandoval-Castilla et al., 2010). Moreover, there was a significant decrease in droplet size of the beads that combined the emulsification system and ionic gelation strategy. This reduction in size was also observed by Beldarrain-Iznaga et al. (2020) and Lu et al. (2019), who attributed this effect of size reduction

Table 1
Water activity and moisture content of GPE-loaded beads influenced by emulsification process.

Parameter	Sample			
	ALG	PEC	ALG - DE	PEC - DE
A_w at 25 °C	0.40 ± 0.01^{b}	0.42 ± 0.01^{a}	0.37 ± 0.02^{c}	0.43 ± 0.01^{a}
Moisture (%)	6.7 ± 0.9^{b}	9.1 ± 1.1^{a}	2.9 ± 1.3^{d}	4.7 ± 1.5^{c}

[a-d]Different letters in the same line indicate a significant difference ($p < 0.05$) using Turkey test.

Table 2
Average diameter (μm) and polydispersity index (PdI) of GPE-loaded beads.

Sample	Diameter (μm)	PdI
ALG	17.1 ± 1.2[b]	0.27 ± 0.02[b]
PEC	21.9 ± 1.7[a]	0.30 ± 0.03[a]
ALG – DE	11.1 ± 1.5[d]	0.31 ± 0.03[a]
PEC – DE	15.3 ± 1.1[c]	0.33 ± 0.02[a]

[a-d]Different letters in the same column indicate a significant difference ($p < 0.05$) using Turkey test.

to the formation of a more cohesive surface structure due to the interaction between the emulsion, calcium ions, and polymer chains, which could compress the hydrogel bead structure. In addition, the presence of phenolic acids in GPE could lead to the formation of smaller droplets as they reduce the interfacial tension between the aqueous phase and the flexible interfacial formation, thus acting as co-surfactants and co-solvent (Fasolin, Santana, & Cunha, 2014).

3.3. Morphology

Fig. 1 shows the morphology of the GPE-loaded beads.

Optical microscopy photographs revealed that the alginate beads prepared by ionic gelation exhibit a regular, round and spherical shape (Fig. 1a and Fig. 1c), whereas the pectin beads have a slightly irregular shape (Fig. 1b and Fig. 1d). One possible explanation is that this irregularity is due to the weaker mechanical stability of the Ca-crosslinked pectin-bead network compared to alginate (Fasolin et al., 2014). Similar trends were reported by Belščak-Cvitanovic et al. (2016) with pectin beads of dandelion polyphenols and ß-carotene generated by ionotropic gelation. These authors showed a lower regularity in spherical form for particles using pectin than particles with alginate.

The results also showed that the emulsion droplets were multinucleated and had defined walls, which should ensure better protection to the grape peel bioactive compounds. Furthermore, the integrity and tightness of the coating wall which can be observed by perfectly enclosing the core material, leading to a gradual diffusion of the core material from the hydrogel beads.

3.4. Anthocyanin content, total phenolic compound content, and antioxidant capacity of GPE-loaded beads

The encapsulation technique using ionic gelation is an effective way to prevent the degradation of bioactive compounds that can occur due to their interaction with temperature/pH variations, and light/oxygen exposure (Fasolin et al., 2014). Anthocyanin content, total phenolic compound content, and antioxidant capacity are shown in Table 3.

Table 3 shows that the parameters of total anthocyanins, total phenolic compound content, and antioxidant activity decreased significantly in the emulsion (GPE-EM) compared with GPE. This effect could be related to the preparation conditions of the emulsions, such as contact with oxygen and the use of an ultrasonic sonicator, which generates cavitation bubbles that collapse and produce high local heat that could degrade some of the phenolic compounds present in the GPE due to their instability under these conditions (Wang et al., 2017). This effect was also observed by de Moura et al. (2018).

According to Table 3, PEC and ALG showed higher losses of monomeric anthocyanins during the encapsulation process than PEC-DE and ALG-DE beads. This loss of anthocyanins may be attributed to the high

Table 3
Anthocyanin content, total phenolic compound content, and antioxidant capacity of GPE-loaded beads.

Sample	Total monomeric anthocyanin (mg 100 g^{-1})	Total phenolic content (mg GAE 100 g^{-1})	Antioxidant activity (mg TEAC 100 g^{-1})
GPE	1048.5 ± 0.10[a]	978.2 ± 0.10[a]	2016.9 ± 0.80[a]
ALG	671.0 ± 0.66[e]	143.6 ± 0.01[e]	306.4 ± 1.30 [e]
PEC	482.1 ± 0.92[f]	63.2 ± 0.01[f]	168.2 ± 0.40[f]
GPE - EM	987.2 ± 0.44[b]	831.7 ± 0.07[b]	1787.4 ± 0.94[b]
ALG - DE	878.6 ± 0.84[c]	768.3 ± 0.02[c]	1353.9 ± 0.60[c]
PEC - DE	750.3 ± 0.70[d]	456.2 ± 0.04[d]	653.1 ± 0.70[d]

[a-e]Different letters in the same column indicate a significant difference ($p < 0.05$) using the Turkey test.

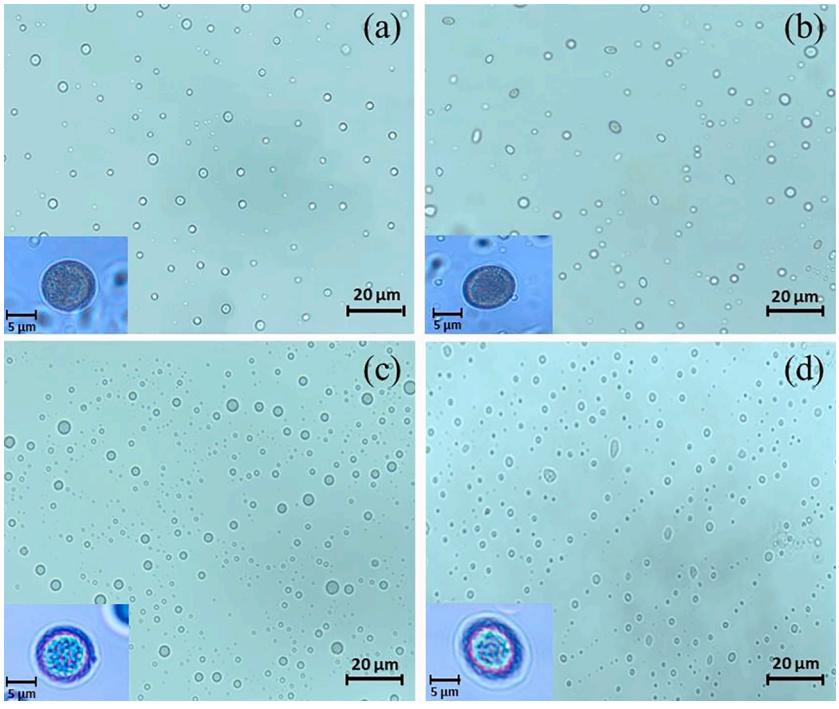

Fig. 1. Morphology of: (a) ALG, (b) PEC, (c) ALG-DE and (d) PEC-DE.

porosity of the beads and the easy diffusion of these bioactive compounds under hydrophilic conditions, especially because of their polar nature (Fasolin et al., 2014).

The emulsion/ionic gelation approach provided higher levels of monomeric anthocyanins content. One possible explanation is that the combined techniques may have reduced the loss of anthocyanins by reducing the effective diffusivity through the microstructures of the hydrogels. In this way, the emulsification process acted as an additional barrier compared to the free anthocyanin extract and affected the polarity of the anthocyanins, hindering diffusion transport from the interior to the surface of the beads. These results are in accordance with da Silva et al. (2019).

Antioxidant capacity analysis and total phenolic content followed the same trend as monomeric anthocyanin content, as displayed in Table 3. The beads containing only grape peel extract showed the lowest antioxidant capacity and total phenolic content, followed by the samples ALG-DE and PEC-DE. In this way, the antioxidant capacity and phenolic content of the beads were directly related to the emulsification process. Pectin and alginate hydrogel beads with combined techniques were more efficient in incorporating and protecting the anthocyanins, resulting in higher antioxidant capacity and phenolic content values in relation to samples do not use the emulsification process. These results are in agreement with the previous studies that showed that ionic gelation/double emulsion of jussara extract (Carvalho et al., 2019) and *Lactobacillus casei* (Beldarrain-Iznaga et al., 2020) exhibited higher antioxidant activity.

The encapsulation efficiency (EE) of GPE was investigated by comparing the ability of encapsulate or hold the grape peel anthocyanins inside the microcapsules using or not the emulsification process. EE was around 46% (PEC), 64% (ALG), 76% (PEC-DE) and 89% (ALG-DE). Therefore, a possible explanation for the increase in the encapsulation efficiency of PEC-DE and ALG-DE microparticles is that soybean oil interacted with the polymeric network of polysaccharides, strengthening the existing chemical bonds and thus acting as an additional physicochemical barrier that limited the diffusion diffusion rate of anthocyanins and consequently protected them (Liu et al., 2019; Van der Ark et al., 2017). This effect was more pronounced at ALG-DE. Previous researches have suggested that alginate with divalent cations forms stronger egg-box structures than pectin (Fang et al., 2008). Similar results were also reported by Carvalho et al. (2019), Liu et al. (2019) and Beldarrain-Iznagaab et al. (2020).

3.5. FTIR spectra GPE-loaded beads

FT-IR analysis was performed to distinguish possible chemical interactions among GPE and also the respective beads constituents. The FTIR-spectra of the samples were displayed in Fig. 2.

The spectrum of pure pectin (PEC-MP) showed vibrational bands at 3627–2910 cm^{-1} (O—H and C—H stretching). Two bands at 1603 cm^{-1} and 1405 cm^{-1} (C—O—O weaker asymmetric and stronger symmetric), at 1200–1000 cm^{-1} (C—O and C—C of glycosidic bonds and pyranoid rings) and the very complex region below 1000 cm^{-1} so-called "fingerprint" of polysaccharides (Synytsya, Čopíková, Matějka, & Machovič, 2003).

The infrared spectra of neat alginate (ALG-MP) showed characteristic bands of its composition, highlighting the absorption bands at 3650–3018 cm^{-1} (hydroxyl group), 1603 and 1402 cm^{-1}, which were assigned to asymmetric and symmetric stretching of carboxylate salt groups. In addition, the bands centered at approximately 2927 cm^{-1} was attributed to the elongation of C—H bonds of pyranoid-ring carbons, 1085 cm^{-1} (C—O stretching), 1027 cm^{-1} (C—O—C stretching), and 947 cm^{-1} (C—O stretching) were attributed to its polysaccharide structure (Synytsya et al., 2003).

For grape peel extract (GPE) the observed bands, as the O—H stretching at 3354 cm^{-1}, 2852 cm^{-1} attributed to the C—H—bonding axial deformations in aliphatic hydrocarbons, 1402 cm^{-1} corresponds to the C—O deformation of phenols and 1742–1707 cm^{-1} attributed to the C═O groups for aromatic rings. The absorbance at 1047 cm^{-1} was due to the stretching vibration of the C—O—C esters whereas absorbance at 1186 cm^{-1} was ascribed to the stretching of the pyran rings, which are typical of flavonoid compounds (Ahmed, 2015).

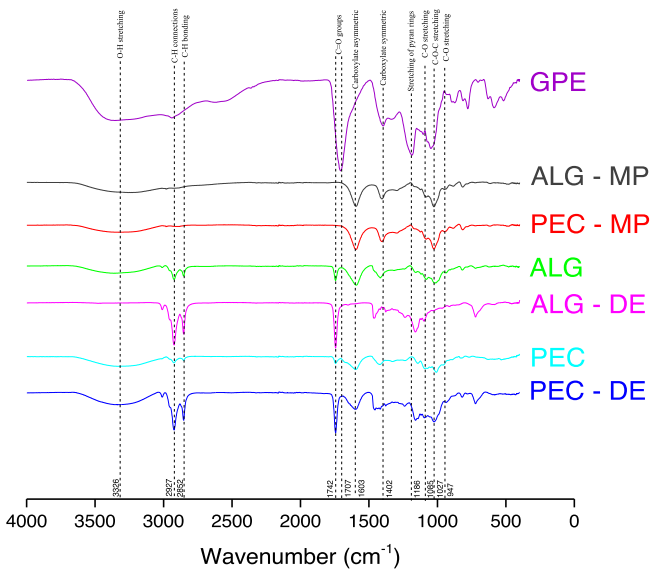

Fig. 2. FTIR spectra of grape peel extract (GPE), neat biopolymers, and GPE-loaded beads. Y-axis: transmittance/a.u.

GPE beads peaks were typical of grape peel extract components. Specifically, the addition of GPE caused the appearance of characteristic bands (2852 cm^{-1}, and 1186 cm^{-1}) as well as the increase in the intensity of these bands when combined emulsification and ultrasonic ionic gelation strategy, also suggesting that ALG-DE retained the bioactive compounds more efficiently. In addition, changes in the wavelength number of the bands related to C═O groups, 1707 to 1742 cm^{-1}, may indicate an interaction between phenols present in GPE. These results are in accordance with Beldarrain-Iznaga et al. (2020) and Silva et al. (2018). The FTIR data strongly support the findings obtained by conducted analyses on bioactive encapsulation parameters, such as anthocyanin content and encapsulation efficiency.

3.6. GPE-loaded beads release profile under simulated gastric and intestinal conditions

Anthocyanins (ATC) are characterized by very low bioavailability and enter the bloodstream rapidly after consumption of a meal rich in these compounds, generally due to their low solubility, low stability, low permeability, an active efflux process, and also gastrointestinal tract metabolism (Pedrali, Barbarito, & Lavelli, 2020).

In this way, encapsulation of bioactive components can improve bioavailability by increasing their water solubility and release in a given environment, leading to better absorption by the human body (Pedrali, Barbarito, & Lavelli, 2020). Therefore, anthocyanin release (Fig. 3) from the microcapsules was determined in simulated intestinal fluid to clarify whether the double emulsification approach can be considered a suitable matrix for stabilizing anthocyanins under detrimental intestinal conditions.

In GPE, 70% of anthocyanins were already detected within the first 10 min. It was also observed that the total anthocyanins content in the gastric fluid decreased from 120 min, probably due to the degradation of anthocyanins caused by exposure to heat (37 °C) for a longer period of time (Pedrali, Barbarito, & Lavelli, 2020). When transferred to the simulated intestinal fluid, anthocyanin release was kept almost constant, until the end of 4 h incubation.

PEC and ALG had a low protective effect on anthocyanins during

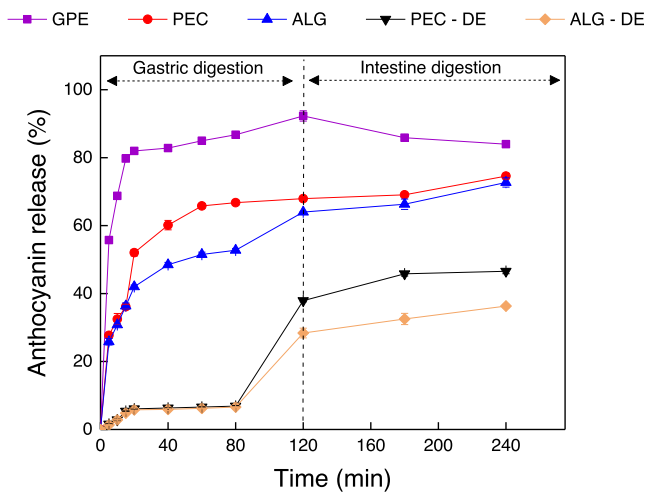

Fig. 3. Release profile of ATC from GPE and GPE-loaded beads in simulated gastric conditions.

gastric digestion, as more than 45% of this pigment was released in the first 20 min. This could be because bioactive compounds are generally small molecules that can easily migrate into the porous structure of hydrogels, resulting in low protection and facilitating release in the stomach (Yao et al., 2018).

On the other hand, microparticles prepared using a double emulsion and ultrasonic ionic gelation showed lower release in simulated gastric fluid than particles prepared with a non-combined strategy. The use of soybean oil to elaborate the double emulsion with grape skin extract before the ultrasonic gelation step contributed to a lower release of anthocyanins because the enzyme lipase is not present in gastric fluid. Thus, the soybean oil protected the GPE by acting as a barrier against the gastric fluid and not fully releasing the bioactive compounds during the gastric digestion (Yao et al., 2018).

In ALG - DE, the anthocyanin content lost in the gastric phase was slightly lower than in PEC-DE. This ALG-DE behavior can be attributed to the stronger physicochemical interaction of the hydroxyl groups in the adjacent chains of the alginate with the polar compounds in the grape peel extract (anthocyanins) and with the associated soybean oil. This resulted in the formation of more complex polymer entanglement and lower porosity due to the addition of soybean oil, which reduced the diffusivity of anthocyanins across the polysaccharide chain interface (Rather et al., 2017). Similar results were pointed out by Beldarrain-Iznaga et al. (2020), Carvalho et al. (2019) and Zhang et al. (2015).

In the simulated intestinal phase (SIF), anthocyanins convert to quinonoid, hemiketal, and chalcone forms and are degraded due to higher pH. Conversion and degradation under intestinal conditions could be a possible reason for the low bioavailability of anthocyanins. Therefore, it would be beneficial for anthocyanins to enter the intestine in their flavylium form (more stable cation). For this reason, the release of anthocyanins in simulated intestinal fluid provides information on whether microparticles are suitable matrices for the stabilization of anthocyanins under intestinal conditions (basic pH) (Betz & Kulozik, 2011).

The release of anthocyanins continued to increase significantly in the beads ($p \leq 0.05$), which is due to the fact that the rate of degradation of alginate is due to an increase in the rate of ß-elimination, which is also the case for pectin, facilitating the lipolysis of soybean oil through the action of pancreatic lipases. This process led to the demulsification of the microcapsules, eventually exposing the anthocyanins to the action of bile salts (He et al., 2017).

However, the ALG-DE microparticles containing soybean oil showed a lower release of anthocyanins during the final stages of digestion, indicating the protective effect caused by the combination of microencapsulation techniques. Similar results were shown by He et al. (2017), where chitosan nanoparticles caused slower release of blueberry anthocyanins than non-encapsulated anthocyanins, indicating that the slow release could reduce the degradation of anthocyanins, leading to bioavailability of these bioactive compounds that are more disponible for absorption in the gastrointestinal tract.

4. Conclusion

The combination of techniques for microencapsulation of grape peel extract influenced the physicochemical, morphological, and biological properties during the digestion process. In particular, ALG-DE showed greater retention of anthocyanins, total phenolic compounds, and antioxidant activity. These results are related to the formation of more complex three-dimensional structures and greater chemical interactions of the alginate/grape skin extract and the oil, resulting in less diffusion of anthocyanins. In addition, smaller particle size, more spherical particles, and greater protection/ bioavailability of anthocyanins during gastrointestinal digestion were evident.

In summary, the combined method for microencapsulation of anthocyanins from grape extract provided additional functional properties to the microparticles and proves to be an interesting strategy for incorporating phenolic compounds into polymeric matrices and directly affects the viability of these compounds during processing, storage, and application, with the advantage of using mild conditions. The proposed technology also highlights the possibility of using agroindustrial by-products of viticulture for the production of extracts rich in anthocyanins, which strengthens the possible application of the combined approach of ionic gelation and emulsification processes.

CRediT authorship contribution statement

Laís Bruno Norcino: Visualization, Writing – review & editing, Conceptualization, Methodology. **Juliana Farinassi Mendes:** Visualization, Writing – review & editing, Conceptualization, Methodology. **Jayne de Abreu Figueiredo:** Data curation. **Natália Leite Oliveira:** Formal analysis. **Diego Alvarenga Botrel:** Project administration, Supervision. **Luiz Henrique Capparelli Mattoso:** Project administration, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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