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Development of alginate beads with encapsulated jabuticaba peel and propolis extracts to achieve a new natural colorant antioxidant additive

Ithiara Dalponte Dallabona [a,*], Gabriel Goetten de Lima [c], Beatriz Isabella Cestaro [d], Ivisson de Souza Tasso [a], Thainnane Silva Paiva [a], Emanuele Joana Gbur Laureanti [e], Luiz Mario de Matos Jorge [f], Bruno José Gonçalves da Silva [d], Cristiane Vieira Helm [d], Alvaro Luiz Mathias [a], Regina Maria Matos Jorge [a,e,**]

[a] Federal University of Paraná, Department of Chemical Engineering, Graduate Program in Food Engineering, Curitiba, PR, Brazil
[b] National Research Center for Forestry, Embrapa Florestas, Estrada da Ribeira, Km 111, Colombo, 83411-000, Brazil
[c] Materials Research Institute, Athlone Institute of Technology, Athlone, Ireland
[d] Federal University of Paraná, Department of Chemistry, Graduate Program in Chemistry, Curitiba, PR, Brazil
[e] Federal University of Paraná, Department of Chemical Engineering, Graduate Program in Chemical Engineering, Curitiba, PR, Brazil
[f] State University of Maringá, Department of Chemical Engineering, Maringá, PR, Brazil

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ABSTRACT

This work aims to encapsulate anthocyanins and phenolic compounds extracted from a native Brazilian fruit peel - jabuticaba (*Plinia cauliflora (Mart.) Kausel)* and propolis from Tubuna (*Scaptotrigona bipunctata*) stingless bees, with great potential benefits for human health. The alginate encapsulation was conducted by the ionotropic gelation through the dripping into the $CaCl_2$ solution. Both raw extracts were characterized by TPC - total phenolic content (Folin-Ciocalteu), AA -antioxidant activity (DPPH and ABTS assays), and TMAC - total monomeric anthocyanin concentration (pH differential method); as well as their resultant mixture (2:1 jabuticaba/propolis). The obtained beads presented highly efficient encapsulation of total polyphenols (~98%) and monomeric anthocyanins (~89%), with spherical morphology and smooth surface obtaining a mean diameter between 200 and 250 μm. In vitro release study showed that JPE/alginate beads were completely disintegrated at pH 7.4 (intestinal pH), but they were resistant to gastric pH (1.2) presenting a slow release of about 40% in 240 min. This is the first report that encapsulates the mixture of jabuticaba and propolis extracts and may contribute to the utilization of a great source of bioactive compounds besides the potential pigment of anthocyanins, an alternative to natural and healthy food/beverage colorants.

1. Introduction

There is a growing interest in reducing the usage of synthetic dyes in food and beverages replacing them to natural colorants; due to the fact that artificial colorants, and their contaminants, may be carcinogenic and/or neurotoxic to humans [1]. In addition, the food industry has been developing food products rich or enriched with natural antioxidants, since they may have a potential role in the prevention of cardiovascular disease, cancer and neurodegenerative disorders [2].

Anthocyanins are natural food colorants with purple, red, or blue colours found in fruits and vegetables which exhibit strong antioxidant activity reporting antiallergenic, antiviral, anti-inflammatory, and vasodilating properties; they are also cytotoxic against cancer cells and inhibit oxidative and inflammatory enzymes [3]. As deeply coloured fruits were found to be a potentially rich source of anthocyanins, phenolic acids and flavonoids; jabuticaba fruits and its extracts are now receiving considerable attention [4].

Jabuticaba (*Plinia cauliflora (Mart.) Kausel*) is a Brazilian native fruit commonly known in South America as "Brazilian berry", which has great economic and commercial potential [5]. Jabuticaba are dark purple spherical berries containing a juicy pulp with a thin and fragile peel; they are slightly acidic and very sweet [6]. This fruit is one of the richest Brazilian sources of anthocyanins based on polyphenolic compounds including anthocyanins, that are primarily concentrated in the fruit peel [4]; this peel is not normally used as a primary source of food but it can be used to produce jams and extracts designed for the beverage industry. Nonetheless, the extract of jabuticaba peel can be used as an active ingredient for food and beverage in order to make it functional; furthermore, it could have a potential effect of replacing artificial dyes such as Red 40, Ponceau 4R, Erythrosine and Bordeaux S. However, the unstable anthocyanin molecule structure limits its appli-

* Corresponding author.
** Correspondence to: R.M. Matos Jorge, Federal University of Paraná, Department of Chemical Engineering, Graduate Program in Food Engineering, Curitiba, PR, Brazil.
E-mail addresses: ithiara@ufpr.br (I. Dalponte Dallabona); rjorge@ufpr.br (R.M.M. Jorge)

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cation due to its degradation in the presence of light and oxygen at pH above 2.

Propolis is a resinous substance with a complex chemical composition with varying colours and consistencies, collected by bees from several vegetal sources. Tubuna (*Scaptotrigona bipunctata*) are native bees from Brazil and popularly known as meliponines or "indigenous stingless bees". The propolis is rich in essential oils and phenolic components also reported to have high levels of antioxidants, antibacterial, anti-inflammatory and anti-tumoral activity [7] with a great variety of applications in food preservation, also beneficial for human health [8]. Even though propolis contains many bioactive compounds (flavonoids, phenolic acids, aldehydes, and ketones), its application as a food additive is also limited by the low oral bioavailability; studies reported that orally administrated propolis is rapidly degraded in the body, especially in the gastrointestinal tract [7]. Furthermore, the strong taste and aroma hinder its direct application; thus, there is an important focus of finding novel approaches that can harness the propolis potential.

Nonetheless, an effective strategy to preserve the health beneficial properties of natural sources is incorporating their extracts rich in polyphenols into polymer matrices for the improvement of stability and bioavailability [9]; moreover, this protection can mask its strong odours or astringent flavours [10]. The encapsulation can improve the delivery systems, offering prolonged and controlled release of food ingredients.

Materials used for encapsulating bioactive compounds include the alginate polymer, which is biodegradable, biocompatible, and has non-toxic nature [11]. Alginate biopolymer is produced naturally by brown sea algae and its molecular structure is formed by a linear binary copolymer of 1 → 4 linked α-D-mannuronic acid (M block) and β-L-guluronic acid (G block) residues in varied proportions. Alginate is an FDA approved polysaccharide used for a variety of applications as outlined by Fernando et al. [12]. Alginate capsules formation occurs in the presence of divalent cations in a process known as ionotropic gelation. The free carboxylic groups react with Ca^{2+} bridging the gap between the two polymer chains (gulunorate or G block and mannuronate or M block), in order to stabilize the network and form stable gel capsules [13]. Tough, this method can be performed with a simple dripping tool like a pipette or syringe, they can also have a more complex methodology. Besides its easy execution, this technique avoids high temperatures and organic solvents and does not require specific equipment for its implementation [14]. Therefore, it provides a good approach for the encapsulation of highly reactive compounds, such as anthocyanins and phenolic compounds. Besides, these capsules or beads could be used directly as an ingredient or could be incorporated in a subsequent technological process with a huge potential for industrial applications [15].

To the best of our knowledge, it is the first time jabuticaba peel extract, rich in anthocyanins, is combined with the extract of native Tubuna stingless bee propolis and, the resulting extract mixture is encapsulated by ionotropic gelation in alginate, enabling its use as a natural colorant additive and source of antioxidants. Alginate encapsulation is expected to protect against pigment degradation of anthocyanins, maintaining their characteristic colour, as well as protecting phenolic compounds from propolis extract, ensuring their stability until its final release. The extracts were characterized for antioxidant activity (AA), monomeric anthocyanin concentration (TMAC), and total phenolic concentration (TPC). The volatile compounds profile was investigated by gas chromatography coupled with mass spectrometry (GC–MS). Additionally, we also performed kinetic studies of anthocyanins release profiles from the beads (polymeric matrix) in simulated gastrointestinal pH conditions.

2. Materials and methods

2.1. Chemicals

All the chemicals and reagents were of analytical grade. Gallic acid, Folin-Ciocalteu, ABTS, DPPH, and Trolox reagents were purchased from Sigma-Aldrich (St. Louis, USA). Sodium alginate with M/G ratio and viscosity of 1.61 and 50–400 mPa.s (1%, water, 25 °C) respectively, was also obtained from Sigma Aldrich. Sodium acetate, sodium carbonate anhydrous, potassium chloride, and potassium persulfate were purchased from Anidrol (São Paulo, Brazil). Ethyl acetate (HPLC grade) was acquired from VWR Chemicals (Briare, France).

2.2. Extracts preparation

The crude Brazilian propolis produced by Tubuna stingless bee was collected in 2019 (March), donated by Jorge's meliponary located near the reserve Bosque II, a native Atlantic forest (GPS coordinates: −23.4318698 and −51.9440141) in Maringá (south of Brazil) and extracted through a series of steps. Briefly, the raw propolis was grounded using a manual mill (Botini, Brazil) and 20 g of this powder was mixed to ethanol solution 70% (v/v) and maintained under dark conditions for 48 h; the propolis powder to solvent ratio was 1:25 (w/v). Then, after maceration, the crude extract was submitted to the ultrasonic bath (1440 DA Biodont, Brazil), with a heating frequency of 40 kHz for 1 h. The suspension was cooled (18 °C) and filtered through Buchner with a glass microfiber filter under vacuum. The final filtrate was evaporated and concentrated on a rotary evaporator (Fisatom, Brazil) at 40 °C under reduced pressure, resulting in 50 mL of crude propolis extract (PE), it was stored in the dark at −20 °C for further analysis.

Jabuticaba Sabará fruits (*Plinia cauliflora*) were acquired from a local fruit and vegetable market center (CEASA-Curitiba, Brazil). The fruits were washed with running water and manually depulped. The peel was freeze-dried (Liotop L101, Brazil) at −54 °C and 20 μHg until a constant weight was reached. Using 652.0 g of fresh peel, it was obtained 114.8 g of freeze-dried material. Afterward, 20 g of freeze-dried jabuticaba peel was added to an acidified hydroalcoholic 70% (v/v) solution (final pH 2.4) using a fruit peel to solvent ratio of 1:25 (w/v). This extract was macerated for 48 h under refrigeration with protection from light with further sonication for 1 h. The final filtrate was concentrated on a rotary evaporator (Fisatom, Brazil) at 40 °C under reduced pressure, and resulted in 200 mL of crude jabuticaba extract (JE); it was stored in an amber bottle at −20 °C for further analysis.

The jabuticaba extract (JE) and propolis extract (PE) were combined in a volume ratio of 2:1 (jabuticaba: propolis extracts) to obtain the extract mixture (JPE).

The proximate composition of freeze-dried jabuticaba peel was determined by analyses of moisture, total protein, total lipids, ash, and total dietary fiber. Total protein and ash were determined according to the AOAC Official Methods 991.20 (2000) and 940.26 (2005), and total lipids by Bligh & Dyer method (AOAC Official Method 920.39.C (2005)). The dietary fiber was performed by enzymatic-gravimetric method (AOAC Official Method 985.29) using the Sigma Aldrich TDF-100A kit. The total carbohydrate was calculated by difference (100- %moisture - %protein - %ash - %lipids).

2.3. Total phenolic content (TPC)

The total polyphenols content (TPC) of the extracts was determined by the Folin-Ciocalteu method [16] with minor modifications. Extracts (0.2 mL) were mixed with 0.5 mL of the Folin-Ciocalteu reagent (2 N), 2 mL of 15% (w/v) Na_2CO_3 solution and 5 mL of distilled water. The absorbance readings at 760 nm were made after 120 min of incuba-

tion in the dark and a standard curve was prepared with gallic acid (1.5–10.0 mg/mL), expressing the results as mg gallic acid (GA)/g of dried sample.

2.3.1. Chemical profile

JE and PE aqueous extracts and JPE (2:1 v/v) mixture were further processed by liquid-liquid extraction for GC–MS analysis. The extract (585 μL) was added to a 2 mL vial, along with 175 μL of ultrapure water and 450 μL of ethyl acetate. The mixture was agitated by vortex for 2 min, and after phase separation, the top organic layer was collected for injection in the chromatographic system. The system used for qualitative GC–MS analysis consisted of a GCMS2010 Plus gas chromatography coupled to a TQ8040 triple quadrupole mass spectrometer (Shimadzu, Japan), equipped with a ZB-5MS column (30 m × 0.25 mm ID, 0.25 μm film thickness) and AC 5000 autosampler. For the method parameters, a sample volume (1 μL) was injected using split injection (split ratio 1:10), using helium as the carrier gas, with a flow rate of 1 mL/min. Injector temperature was set to 250 °C and column heating program was conducted as follows: 50 °C, held for 4 min, 10 °C /min to 280 °C, held for 3 min. Transfer line temperature was set to 300 °C and the ion source temperature was set to 250 °C. Ionization was achieved by electron impact (70 eV) and the acquisition was conducted in full scan mode, at a scanning range of 50–650 *m/z*. Compounds were then identified according to NIST 14 Mass Spectral Library.

2.4. Encapsulation of extracts

Beads were prepared by ionotropic gelation by dispensing it as liquid droplets according to our previous reported methodology [17]. The JPE extract was added to a 3% (w/v) sodium alginate solution; homogenized in a magnetic stirrer overnight and subjected to the dripping methodology (Fig. 1). The resulting solution was dripped by using a peristaltic pump through a 200 μL micropipette tip (inner diameter of 1.2 mm) into a 4 wt% $CaCl_2$ solution at the speed of 100–130 drops/min; this step was performed at room temperature under gentle stirring. The suspended beads were maintained in the $CaCl_2$ to harden for 30 min; thereafter, the particles were collected by a sieve and stored for further TPC and anthocyanins determination. The particles were washed several times using distilled water to remove unbound calcium chloride from the bead surface, the JPE/alginate beads were then oven-dried at 40 °C for 24 h.

2.5. Evaluation of antioxidant activity (AA)

The assessment of in vitro antioxidant activity (AA) from JE, PE, and JPE extracts encapsulated and non-encapsulated were performed through the evaluation of free radical capture DPPH [18] and ABTS [19]. Briefly, 100 μL of sample extract and 3.9 mL of DPPH methanolic solution (0.06 mM) were added and mixed thoroughly and the absorbance readings were made at 515 nm after 30 min of reaction using a UV–vis spectrophotometer (Shimadzu, UV-1800). For the $ABTS^{*+}$ radical assay, ABTS salt solution (7 mM) was mixed with the potassium persulfate solution (140 mM), which was kept in the dark at room temperature for 16 h to allow complete radical generation. After 16 h, sodium acetate buffer (80 mM) was used to adjust the absorbance of the $ABTS^{*+}$ reagent. Thereafter, 3 mL of $ABTS^{*+}$ reagent was added to 30 μL of sample and the mixture was kept in the dark for 30 min and the absorbance was measured against the sodium acetate buffer at 734 nm. All results were performed by triplicate experiments and expressed as Trolox equivalents (μM TEAC/g), calculated from a standard curve.

2.6. Total monomeric anthocyanin concentration (TMAC)

Total monomeric anthocyanin content (TMAC) was determined using the pH differential method [20]. This method consists of preparing two sample dilutions in two systems: potassium chloride buffer pH 1.0 (0.025 mol/L) and sodium acetate buffer pH 4.5 (0.4 mol/L). Dilutions were kept resting for 15 min, and the absorbance readings were further performed at the maximum absorbance wavelength λ_{max} at 700 nm (to correct for haze) against a blank cell filled with distilled water in a UV–Vis spectrophotometer. The absorbance of the diluted sample (A) was determined following Eq. (1):

$$A = \left(A_{\lambda max} - A_{700}\right)_{pH\ 1.0} - \left(A_{\lambda max} - A_{700}\right)_{pH\ 4.5} \quad (1)$$

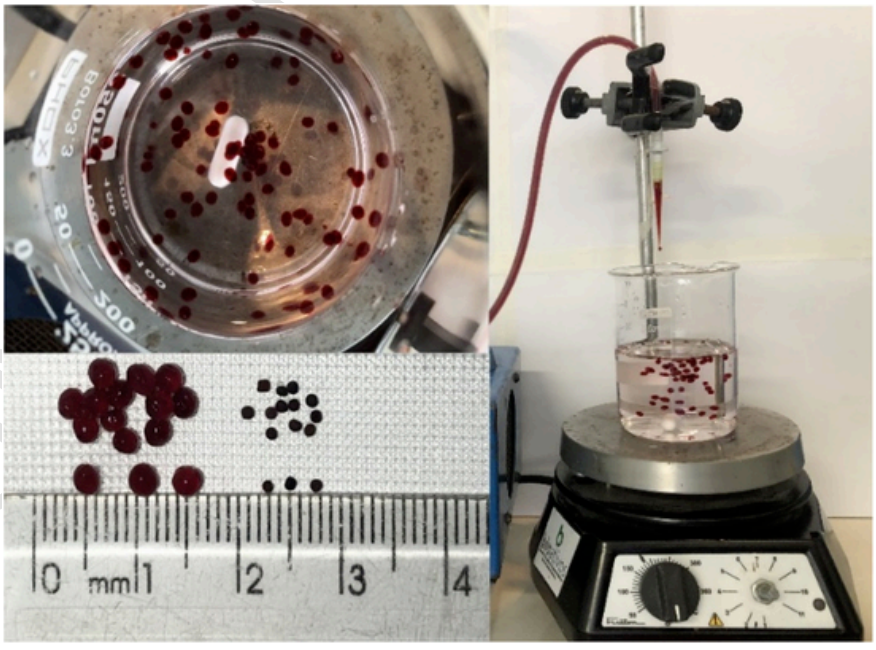

Fig. 1. Encapsulation of bioactives from the JPE mixture extracts of jabuticaba and propolis (2:1).

The TMAC in the original sample was calculated using Eq. (2):

$$\text{Total monomeric anthocyanin concentration (mg/L)} = (A \times MW \times DF \times 1000) / (\varepsilon \times 1) \quad (2)$$

where MW is the molecular weight (MW = 449.2 g/mol) and ε is the molar absorptivity (26,900 L/mol.cm) of the predominant anthocyanin - cyanidin-3-glucoside - and DF is the dilution factor, assuming a path-length of 1 cm.

2.7. Characterization of encapsulated JPE/alginate beads

The mean diameter and weight of 50 JPE/alginate beads were determined using a digital caliper (Mitutoyo Corporation, São Paulo, Brazil) and an analytical balance (Shimadzu, United States), respectively. The bead morphology was characterized using a scanning electron microscope TESCAN VEGA LMU (Cambridge, UK) at an accelerating voltage of 8.0 kV after being coated with gold. The IR spectra were obtained using a spectrometer FT-IR (Vertex 70, Bruker, Germany) in conjunction with attenuated total reflection (ATR). The spectra were recorded in the wavenumber range of 4000–400 cm^{-1} (1 cm^{-1} resolution).

2.8. Encapsulation efficiency (EE)

For the encapsulation efficiency, both TPC and TMAC were evaluated. For anthocyanins encapsulation efficiency, two methods were employed: (1) a method based on the complete dissolution of the beads in 5% (w/v) sodium citrate solution in a Dubnoff shaker for 2 h at 37 °C and 100 rpm; and (2) an alternative method based on the concentration of anthocyanin not encapsulated. EE (%) was calculated by Eq. (3):

$$EE\,(\%) = \frac{\text{mass of loaded anthocyanins}}{\text{mass of initial anthocyanins used}} \times 100 \quad (3)$$

where the mass of loaded anthocyanins is the amount of anthocyanins determined on the solution of sodium citrate and the initial mass of anthocyanins used is related to the anthocyanin on the extract dissolved prior to the alginate solution. The alternative method for encapsulated anthocyanin concentration was indirectly assessed by measuring the anthocyanin content in the $CaCl_2$ solution (i.e. amount not entrapped), also determined by the pH differential method (Section 2.7). In this case, the EE (%) was determined by Eq. (4):

$$EE\,(\%) = \frac{\text{mg anthocyanin extract/mL} - \text{mg anthocyanin not encapsulated/mL}}{\text{mg anthocyanin extract/mL}} \times 100 \quad (4)$$

The TPC content was monitored on the $CaCl_2$ solution and the encapsulation efficiency was determined following the Eq. (4), considering the total phenolic content on the extract (mg GAE/mL) and the ones not encapsulated (mg GAE/mL).

2.9. TMAC and TPC release studies

For extract release from synthesized alginate beads, the system was exposed to simulated gastric (SGF) and intestinal (SIF) pH conditions, without adding digestive enzymes [21]; in which about 5 g of JPE/alginate beads were immersed in glass beakers containing 10 mL of 0.1 N hydrochloric acid (pH 1.2, similar to gastric fluid) and a potassium chloride buffer (pH 7.4, similar to small intestine fluid). The samples were incubated for 240 min in a shaker (NT 232 model, Novatécnica, Brasil) at 37 °C and mild agitation of 100 rpm. At specific time intervals (0, 30, 60, 120, and 240 min) the supernatant solution was analysed by the pH differential and Folin Ciocalteu methods. The amount of TPC and TMAC in the samples was determined, and the release of beads was calculated as a percentage value.

2.10. Statistical analysis

The results were statistically evaluated using Tukey's test at the 95% level of significance, using the Statistica® software version 12 (StatSoft Inc., Tulsa, USA).

3. Results and discussion

To understand the potential benefits of jabuticaba peel waste, we first characterize and analyze its peel. The average weight percentage of fresh peel in relation to the whole fruit was about 32.1% and the moisture content of fresh jabuticaba peel was 82.13 ± 1.01%. The proximate composition of the freeze-dried jabuticaba peel is presented in Table 1, and carbohydrates are the main constituents (~88 wt%); in which almost 20% is dietary fiber. Proteins (3.44%), ashes (2.59%), and lipids (1.14%) are the constituents in smaller proportions. These values found in peel were significant indicating that this residue has great nutritional potential.

3.1. Total phenolic content (TPC) and antioxidant activity (AA)

In this study, the obtained values for total phenolic concentration (TPC) and antioxidant activity (AA) are presented in Table 2. The high TPC for JE extract 112.2 ± 9.4 mg of GAE/g freeze-dried peel is between the values reported in the literature with yields from 23.73 to 556 mgGAE/g dry peel [22,23]. For the propolis extract (PE), the TPC was 6.8 ± 0.1 mg GAE/mL of extract, which is considered low since Fianco et al. [24] reported phenolic concentration of 6.06% or 60.6 mg GAE per g of extract for propolis produced by *Scaptotrigona bipunctata* bee. Differences may be attributed to the chemical composition of propolis that can vary significantly depending on the bee collector preferences, geographic regions, as well as the time of collection.

The JPE mixture presented 20.0 ± 0.6 mg of GAE/mL, which was higher than the sum of both JE and PE extracts individually. By analysing the Tukey's test, there was a significant difference ($p < 0.05$) between JPE responses for total phenolic concentration and the sum of JE and PE individual responses. Therefore, it can be suggested that both extracts have compounds that do not disrupt one another, on the contrary, their combination enhances the total phenolic content. This premise is only valid because we use the same indirect method of determination for total phenolic concentration (Folin-Ciocalteu) for all extracts. Another possibility for the increased result could be attributed to the fact that the mixture can hydrolyze some heterosides and exposes some of the OH groups and, consequently, these free groups can interact with Folin reagent increasing the values found for the mixture.

The JE presented high antioxidant activity (AA) by ABTS and DPPH methods. The ABTS assay presented a value of 1486.3 ± 8.1 μmol TEAC/g for freeze-dried peel, reports with jabuticaba freeze-dried

Table 1
Proximate composition of the jabuticaba peel (g/100 g).

Parameter evaluated	Mean value [a] ± SD
Moisture content	4.59 ± 0.31
Ash	2.59 ± 0.14
Total lipids	1.15 ± 0.09
Crude protein	3.44 ± 0.07
Total carbohydrates	87.89
Total dietary fiber	19.93 ± 0.40

[a] On dry matter basis.

Table 2
TPC and AA by DPPH and ABTS for pure jabuticaba extract (JE), propolis extract (PE) and jabuticaba and propolis extracts (JPE).

Extract	TPC (mg GAE equivalent)	AA on ABTS (μmol TEAC equivalent)	AA on DPPH (μmol TEAC equivalent)
Jabuticaba (JE)	112.3 ± 9.4 mg/g [a] 9.34 ± 0.78 mg/mL [b]	1486.3 ± 8.1 μmol/g [a] 123.6 ± 0.7 μmol/mL [b]	344.7 ± 6.2 μmol/g [a] 28.7 ± 0.4 μmol/mL [b]
Propolis (PE)	18.6 ± 0.3 mg/g [a] 6.76 ± 0.11 mg/mL [b]	17.1 ± 1.4 μmol/g [a] 6.21 ± 0.51 μmol/mL [b]	25.7 ± 0.8 μmol/g [a] 9.35 ± 0.3 μmol/mL [b]
JPE extract	143.5 ± 4.8 mg/g [a] 20.0 ± 0.7 mg/mL [b]	1641 ± 5.5 μmol/g [a] 228.6 ± 5.5 μmol/mL [b]	351.9 ± 4.5 μmol/g [a] 49.0 ± 0.6 μmol/mL [b]

[a] mg GAE (gallic acid equivalent) or μmol TEAC (Trolox equivalent)/g of dry matter.
[b] mg GAE or TEAC per mL of obtained extracts.

peel exhibited a range from 223.1 [25] to 9458 [23] μmol TEAC/g; therefore, the geographic and time collection also can contribute to variations to antioxidant activity. The PE propolis extract revealed an AA by ABTS of 6.21 ± 0.51 μmol TEAC/mL and the mixture JPE extract presented 228.6 ± 5.5 μmol TEAC/mL of extract.

For the DPPH results, jabuticaba (JE) and the propolis (PE) extracts presented 344.7 ± 6.2 μmol TEAC/g and 25.7 ± 0.8 μmol TEAC/g of raw material, respectively. Finally, the JPE extract achieved antioxidant activity of 49.0 ± 0.6 μmol TEAC/mL of extract by DPPH. It is possible to observe (Table 2) that, even though JE and JPE exhibited good antioxidant activities by both DPPH and ABTS methods, the ABTS radical scavenging values were higher than the DPPH. This can be attributed to the high-pigmented and hydrophilic antioxidants which are better reflected by ABTS assay than DPPH assay [26].

It is worth mentioning that, when the extracts were mixed, their antioxidant activity (AA) response was higher than the sum of their individual responses, JE and JP. This could be explained by the increase in total phenolic content (TPC) when JE and PE were combined (2:1), consequently, their JPE antioxidant activity also increased.

Another reason for the enhanced antioxidant activity could be due to a synergic combination between extracts JE and PE. However, the mechanisms responsible for synergistic antioxidant activity are not yet totally understood due to the complex nature of the plant extracts. The compound α-tocopherol has been reported to have a synergistic effect with other antioxidants, such as polyphenols [27]. Furthermore, the synergy between phytochemicals, mainly polyphenols, is demonstrated to show different levels from increasing antioxidant activity to reducing tumor incidence and growth [28]. According to Luís et al. [29] the most important factor to the existence of synergistic effects in mixtures is the total number of aromatic rings. The aromatic rings confer to the compound their resonance structure, due to the delocalization of the electrons in the molecules.

Notably, the JPE is a complex mixture, and in complex mixtures, the combined effect of the phytochemicals can result in a biological effect which is higher or lower than the cumulative effect of each single compound. In regard to crude extracts of food plants or herb products, only a few information exists about the interaction between constituents, and up to now, no studies reported the interaction between propolis and jabuticaba extracts; therefore, additional data are needed to fully evaluate the effects of synergism occurrence.

3.2. Investigation of volatile compounds

Volatiles plays a key role in producing the flavour of natural products, and their investigation is important to ensure processing procedures that result in high quality of aroma to their final product. Additionally, terpenes have been reported to present important antioxidant activity and have demonstrated to possess a wide range of biological activities for human health protection [30]. Moreover, volatile compounds may influence the biological activity of extracts as reported by other studies, volatile terpenes have a synergistic cytostatic effect together with the phenolic compounds, against tumor cell lines, for example. They help in generating additional radicals, after penetrating the cells, by interacting with reactive oxygen species and acts as pro-oxidants, preventing cytotoxic effects [31]. For these reasons, the extracts volatile profile was investigated using the GC–MS assay.

For the GC–MS identified compounds, although a direct quantitative analysis of this volatiles is not simple due to the usage of different isolation procedures and quantification methods, the analysis performed herein was based on the relative frequency of compounds. Among the identified compounds in the propolis extract (PE), terpenes, alcohols, acetophenone and benzopyrone were found to be the major volatile components. The terpenoids class - mainly the mono and sesquiterpenoids - are considered the principal volatile constituents of (geo)propolis produced by stingless bee [8]. The identified compounds of this class for Tubuna propolis were the monoterpenes - 3-carene (6.30%), α-pinene (4.53%), sobrerol (2.80%), terpinen-4-ol (2.67%) - and sesquiterpenes, such as aromadendrane-4,10-diol (3.08%), oplopanone(2.88%) and α-nerolidol (2.42%).

The (+)-α-pinene (4.53%) is responsible for a piney odour, while 3-carene (6.30%) for a sweet and pungent. The vanillin (2.20%) and α-nerolidol (2.42%) give the sweet and floral odours. Also, limonene (1.41%) has citrus and terpene odour description [32]. Among the alcohol class, 2-phenyl ethanol (3.33%) is responsible for the honey and flowery flavour [33]. The benzopyrone identified was the coumaran (dihydrobenzofuran) also reported by Torres et al. [34].

It is important to emphasize that so far, this is the first study reporting the presence of some compounds in *Scaptotrigona bipunctata* stingless bee propolis, such as sobrerol, oplopanone, α-ionol and car-3-en-5-one. The composition of propolis may vary according to floral and geographical origin (see Supplementary material A), and those mainly depend on the qualitative and quantitative variations of its characteristic chemical constituents, which are provided by botanical sources. When comparing the results obtained herein with literature, it is possible to state that there is a wide variation in the phenyl composition of stingless bee from Brazil and the world (see Supplementary material A); therefore, it is necessary to treat each propolis as an individual product (Table 3).

Among the identified compounds on freeze-dried jabuticaba peel extract, terpenes are the predominant class of phenolic compounds identified by GC–MS assay, and they are the main contributor to the flavour of jabuticaba fruits [33]. The diterpene phytol, followed by the sesquiterpenes 6-epi-shyobunol (5.98%), τ-muurolol (5.95%), τ-cadinol (3.42%) and δ-cadinene (2.95%) were the most abundant of terpenes identified. Linoleic acid (7.34%) is an important organic acid and, besides of phytol (8.57%), they constitute the major volatile compounds. From within the phenolics, α-tocopherol (3.93%) is an essential micronutrient that is present in the jabuticaba peel and is a source of lipid-soluble vitamin E. The volatile compounds α-muurolene (1.09%), γ-cadinene (0.56%), δ-cadinene (2.95%) were reported to be responsible for the woody [32,33], and τ-muurolol (5.95%) for the spicy odours [33]. Terpenes are also considered to be a major contributor

Table 3
Volatile identified compound by GC–MS of propolis extract from Tubuna bee (PE).

Compound	Rt (min)	Molecular formula	CAS #	%A	Mass spectrum ions (m/z) [b]
Terpenes					
Monoterpenes					
(+)-α-Pinene	7.073	$C_{10}H_{16}$	7785-70-8	4.53	93, 91, 92 (136
(−)-β-Pinene	8.034	$C_{10}H_{16}$	18172-67-3	1.64	93, 91, 69, 77, 79(136)
3-Carene	8.661	$C_{10}H_{16}$	13466-78-9	6.30	93, 91, 79, 77, 92(136)
o-Cymene	8.978	$C_{10}H_{14}$	527-84-4	1.06	119, 91 **134**, 117, 11
(+)-Limonene	9.073	$C_{10}H_{16}$	5989-27-5	1.41	68, 93, 67, 79, 94(136)
Isoborneol	11.626	$C_{10}H_{18}O$	124-76-5	0.50	95, 67, 69, 110 121, 136(15
Terpinen-4-ol	11.756	$C_{10}H_{18}O$	562-74-3	2.67	71, 93, 111, 136, **15**
p-Cymen-8-ol	11.831	$C_{10}H_{14}O$	1197-01-9	1.44	135, 91 117, 65 **150**
α-Terpineol	11.976	$C_{10}H_{18}O$	98-55-5	1.02	59, 93, 121, 136, 81(154)
(−)-Verbenone	12.183	$C_{10}H_{14}O$	1196-01-6	1.73	107, 91 135, 80 **150**
p-Menth-2-en-1,4-diol	13.443	$C_{10}H_{18}O_2$	374169 [a]	2.33	109, 81 127, 91 119(17(
car-3-en-5-one-	13.690	$C_{10}H_{14}O$	81800-50-2	2.44	**150**, 79 135, 107, 91
Sobrerol	14.685	$C_{10}H_{18}O_2$	498-71-5	2.80	109, 59 79, 137 152(17(
1,2,4-Trihydroxy menthane	16.113	$C_{10}H_{20}O_3$	22555-61-9	2.47	109, 127, 81 71, 145(18
2-Acetoxy-1,8-cineole	17.835	$C_{12}H_{20}O_3$	72257-53-5	2.87	71, 108 93, 67, 81(212)
Sesquiterpenes					
α-Nerolidol	16.968	$C_{15}H_{26}O$	40716-66-3	2.42	69, 93, 107, 136, 121(22
4,10-Aromadendranediol	18.940	$C_{15}H_{26}O_2$	384016 [a]	3.08	119, 107, 93 79, 162(23
Oplopanone	19.060	$C_{15}H_{26}O_2$	1911-78-0	2.88	135, 153, 71 111, 107(23
Isoaromadendrene epoxide	20.488	$C_{15}H_{24}O$	159366 [a]	1.08	107, 81 91, 121 71(220)
Phenolic compounds					
Phenol	8.000	C_6H_6O	108-95-2	0.84	**94**, 66, 65
o-Guaiacol	10.093	$C_7H_8O_2$	90-05-1	0.70	109, **124**, 81 53
3-Ethylphenol	11.425	$C_8H_{10}O$	620-17-7	2.51	107, **122**, 77 79
Vanillin	14.875	$C_8H_8O_3$	121-33-5	2.20	**152**, 151, 109, 81, 123
4-Acetylphenol	15.358	$C_8H_8O_2$	99-93-4	5.46	121, 93, **136**, 65
Dihydroconiferyl alcohol	17.970	$C_{10}H_{14}O_3$	2305-13-7	3.35	137, **182**, 122, 106, 91
Alcohols					
2-Ethylhexanol	9.030	$C_8H_{18}O$	104-76-7	0.60	57, 55, 70, 83, 98(130)
Benzyl alcohol	9.138	C_7H_8O	100-51-6	4.78	79, **108**, 77, 107
2-Phenylethanol	10.585	$C_8H_{10}O$	60-12-8	3.33	91, 92, **122**, 65, 57
Dimethylhexynediol	13.190	$C_8H_{14}O_2$	142-30-3	1.34	109, 81, 127, 79, 91(142)
Others					
Coumaran	12.245	C_8H_8O	496-16-2	7.45	**120**, 91, 119, 65
α-Ionol	17.143	$C_{13}H_{22}O$	25312-34-9	1.69	95, 109, 81, 69, 138(194)
Isobutyl phthalate	20.301	$C_{16}H_{22}O_4$	84-69-5	6.01	149, 57, 104, 223, 167(278)
Not identified				15.0	

Rt: retention time; %A: relative compound area.
[a] Nist number.
[b] Ions listed in decreasing order of intensity; highlighted in bold the molecular ion when present among principle ions; and displayed in brackets (ion) in case the molecular ion is not present among principle ions.

to the typical sweet, subacid and slight resinous flavour of the jabuticaba fruits [33].

The literature already provided information about some of these volatiles, such as: α-pinene, β-pinene, D-germacrene; δ-cadinene, τ-muurolol, phytol, α-muurolene, spathulenol and τ-cadinol. However, we also identified new compounds such as cis-sabinol, shyobunol, 6-epi-shyobunol, car-3-en-5-one, triacetin, oplopanone, longifolenaldehyde and aspidinol (see Supplementary material A).

As previously mentioned, the volatile α-tocopherol was found to present a synergetic effect with other phenolic compounds [27,35], also supporting the synergic combination of these compounds in order to enhance the JPE extract antioxidant activity.

The volatile constituents of the mixture of jabuticaba and propolis extracts (JPE) are reported in Table 5. The JPE presented the majority class of terpene compounds, in which the sesquiterpenes presented higher peak areas, accounting 7.0% for phytol, 4.60% for 6-epi-shyobunol, 4.35% for τ-muurolol and 2.67% for δ-cadinene; whereas phenolic compounds are represented by pyrogallol (8.79%) and α-tocopherol (2.44%). Linoleic acid (8.93%) is also an important compound identified which was also reported in the jabuticaba extract peel [6].

From Table 5 it is observed the enhancement of the relative peak area of some constituents when compared to its relative peak area on individual extracts; is the case of pyrogallol phenolic compound (0.42% on JE and 8.79% on JPE). Such an increase in relative peak area may be due to the degradation of anthocyanins when extract pH is slightly raised. JE was previously acidified (pH 2.4) to guarantee anthocyanin stability, but when mixed to PE, which was not subjected to pH control, the resulting JPE presents an intermediate pH. When pH is raised, an-

thocyanins are converted to its respective chalcone due to the opening of the *pyrylium* ring, which can be further decomposed by B-ring cleavage, resulting in phenolic acids. Sadilova et al. [36] described such degradation for pelargonidin-3-glucoside and cyanidin-3-glucoside, which resulted in the formation of 4-hydroxybenzoic acid and protocatechuic acid, respectively. A parallel can be traced with this study, in which delphinidin-3-glucoside - an anthocyanin which exhibits a pyrogallol moiety in the flavylium B-ring and corresponds to the second major anthocyanin in jabuticaba peel [37] - could be suffering decomposition to produce to gallic acid. This phenolic acid would suffer thermal degradation due to high temperatures [38] in the GC–MS injector, resulting in a pyrogallol peak. The results obtained in this study by GC–MS indicate that a minor degradation of delphinidin-3-glucoside can be observed by JE analysis, due to the presence of a pyrogallol peak, and such degradation is increased in JPE, due to a higher pH, resulting in a more evident pyrogallol peak.

Results obtained by GC–MS further indicate that the higher AA, observed for JPE when compared to JE and PE, is a result of a synergistic combination between compounds present in such extracts, instead of the formation of new antioxidants compounds in the mixture, once there are no additional identified compounds. Similar results were previously reported by Wang et al. [39], concerning distinct phytoextracts mixtures.

3.3. Total monomeric anthocyanin content (TMAC)

The performed spectral scanning of JE and its mixture with propolis (JPE) (Fig. 2) presented its maximum wavelength at 512 nm, corresponding to cyanidin-3-glucoside - the main anthocyanin identified in jabuticaba fruit [23]. The extraction of monomeric anthocyanins from jabuticaba peel (JE) resulted in 1318.5 ± 86.8 mg/L (Table 4) which is equivalent to 11.8 ± 0.8 mg of cyanidin-3-glucoside/g freeze-dried peel. Values reported in the literature have shown lower and higher yields of 1.73, 4.04 and 15.14 mg/g [22,23,25]. For JPE mixture, the monomeric concentration (TMAC) had a high value of 1105.8 ± 42.9 mg/L extract. It is important to mention that the concentration of anthocyanins found within JPE extract can be considered as beneficial for human health [23]. Anthocyanins exhibit strong antioxidant activity which helps to prevent neuronal diseases, cardiovascular illnesses, cancer, diabetes, inflammation, and many other diseases [2]. Furthermore, the high antioxidant activity and total phenolic con-

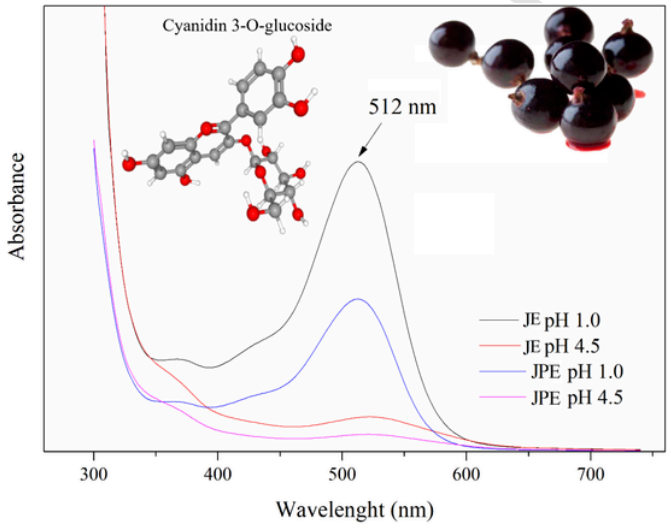

Fig. 2. Spectral characteristics of extracts (cyanidin-3-o-glucoside anthocyanin) in pH 1.0 and pH 4.5 buffers.

Table 4
Volatile identified compound by GC–MS of jabuticaba freeze-dried peel extract (JE).

Compound	Rt (min)	Molecular formula	CAS#	%A	Mass spectr ions (r z)[b]
Terpenes					
Monoterpenes					
o-Cymene	8.978	$C_{10}H_{14}$	527-84-4	0.54	119, 9 **134**, 117, 1
(−)-Cis-sabinol	12.109	$C_{10}H_{16}O$	3310-02-9	0.55	92, 79 134, 8
Car-3-en-5-one	13.690	$C_{10}H_{14}O$	81800-50-2	0.98	119(1 **150**, 7 135, 107, 9
Sesquiterpenes					
D-germacrene	16.075	$C_{15}H_{24}$	23986-74-5	0.12	161, 105, 9 119, 81(20
α-Muurolene	16.261	$C_{15}H_{24}$	10208-80-7	1.09	105, 161, 9 119, 81(20
γ-Cadinene	16.458	$C_{15}H_{24}$	39029-41-9	0.56	161, 105, 119, 9 133(2
δ-Cadinene	16.501	$C_{15}H_{24}$	483-76-1	2.95	161, 119, 105, 134, 2
Elemol	16.868	$C_{15}H_{26}O$	639-99-6	0.72	93, 59 107, 161, 81(22
Spathulenol	17.261	$C_{15}H_{24}O$	6750-60-3	0.61	91, 10 119, 159, 79(22
(+)-Isospathulenol	17.842	$C_{15}H_{24}O$	88395-46-4	1.57	119, 9 105, 159, 162(2
τ-Cadinol	18.030	$C_{15}H_{26}O$	5937-11-1	3.42	161, 9 105, 119, 121(2
τ-Muurolol	18.168	$C_{15}H_{26}O$	19912-62-0	5.95	95, 12 161, 9 81(22
α-Muurolene-14-ol	18.231	$C_{15}H_{24}O$	135118-51-3	1.07	159, 9 117, 105, 81(22
Ent-germacra-4(15),5,10(14)-trien-1β-ol	18.517	$C_{15}H_{24}O$	81968-62-9	1.78	109, 9 79, 15 81(22
6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro-naphthalen-2-ol	18.584	$C_{15}H_{24}O$	189102[a]	1.56	159, 131, 105, 9 **220**
Shyobunol	18.653	$C_{15}H_{26}O$	35727-45-8	0.75	84, 81 67, 12 105(2
Longifolenaldehyde	18.701	$C_{15}H_{24}O$	19890-84-7	1.00	95, 10 81, 79 121(2

Table 4 (*Continued*)

Compound	Rt (min)	Molecular formula	CAS#	%A	Mass spectrum ions (m/z) [b]
Oplopanone	19.060	$C_{15}H_{26}O_2$	1911-78-0	1.10	
Eudesma-4(14),7-dien-1β-ol	19.525	$C_{15}H_{24}O$	192120-23-9	1.09	
6-Epi-shyobunol	19.938	$C_{15}H_{26}O$	69350-61-4	5.98	
Platambin	20.688	$C_{15}H_{26}O_2$	58556-80-2	2.26	
Diterpene					
trans-Geranylgeraniol	18.751	$C_{20}H_{34}O$	24034-73-9	1.18	
Phytol	22.734	$C_{20}H_{40}O$	150-86-7	8.57	
Phenolic compounds					
3-Ethylphenol	11.425	$C_8H_{10}O$	620-17-7	0.50	
Pyrogallol	14.388	$C_6H_6O_3$	87-66-1	0.42	
Aspidinol	21.964	$C_{12}H_{16}O_4$	519-40-4	0.62	
α-Tocopherol	27.108	$C_{29}H_{50}O_2$	59-02-9	3.93	
Esters					
Triacetin	14.015	$C_9H_{14}O_6$	102-76-1	1.28	
Isobutyl phthalate	20.301	$C_{16}H_{22}O_4$	84-69-5	1.77	
Ethyl palmitate	21.611	$C_{18}H_{36}O_2$	628-97-7	1.29	
Hexanedioic acid, bis(2-ethylhexyl) ester	25.156	$C_{22}H_{42}O_4$	103-23-1	1.62	
2-(Dimethylamino)ethyl carbamate	25.693	$C_5H_{12}N_2O_2$	4220-32-0	1.30	
Other compounds					
Coumaran	12.245	C_8H_8O	496-16-2	1.08	
1,6-Anhydro-β-D-glucopyranose	15.977	$C_6H_{10}O_5$	498-07-7	0.20	
2(3H)-benzothiazolone	18.270	C_7H_5NOS	934-34-9	0.46	**151**, 123, 96, 122, 70
Linoleic acid	22.911	$C_{18}H_{32}O_2$	60-33-3	7.34	67, 81, 95, 55, 109(280)
Not identified				30.1	

Rt: retention time; %A: relative compound area.
[a] Nist number.
[b] Ions listed in decreasing order of intensity; highlighted in bold the molecular ion when present among principle ions; and displayed in brackets (ion) in case the molecular ion is not present among principle ions.

tent of the obtained mixture extract JPE are associated with its high anthocyanin content of the jabuticaba extract.

3.4. JPE/alginate bead characterization

The alginate particles had spherical geometry (Fig. 3) with a diameter ranging from 2.0–2.5 mm in the swollen state, and around 1 mm when dried; its particle size is consistent with the size of calcium alginate beads produced by the dripping technique [40]. The droplet size and hence, the final beads, are influenced by the needle/tip diameter of the dripping system, viscosity, and flow rate of the feed material [41].

The SEM photomicrographs exhibited irregular surface after the drying process, presence of cracks but no pores (Fig. 3). According to Jansen-Alvez et al. [42], the presence of cracks can damage the adequate protection of the active compounds, favoring the oxygen transfer, and thus, leading to their degradation. Nonetheless, it is important to mention that the oven-drying is commonly known to produce cracks at crosslinked polymers and alternative freeze-drying is more commonly used; also, their surfaces follow the standard aspect of alginate spheres with a somewhat rough structure.

FT-IR analysis allows identifying functional groups since each functional group absorbs radiation in a characteristic frequency of the infrared spectrum. The infrared spectra of the beads from plain alginate and encapsulated JPE/alginate are displayed in Fig. 4, covering the region from 400 to 4000 cm^{-1}. Characteristics peaks of alginate beads can be observed, whereas the strong absorption band in the region of 3450–3100 cm^{-1} corresponds to the –OH vibration and peaks at 1688–1600 cm^{-1} may also correspond to symmetrical and asymmetrical stretching vibration for the carboxyl ion (COO–); indicating the existence of carboxylic acid, ester, or carbonyl groups. Bands at 1504–1360 cm^{-1} correspond to the C═C of the aromatic ring, and main peaks between 1400 and 1000 cm^{-1} are attributed to C–O–C stretching vibrations.

In particular, anthocyanins as cyanidin compound has an absorption spectra region of 3100–3400 cm^{-1} (O—H symmetric stretching vibration); also others showing at 2900–2840 cm^{-1} (C—H aliphatic), 675–870 cm^{-1} (C—H aromatic) and 1660 cm^{-1} (C═C aromatic) [43,44]; therefore, also confirming the anthocyanins compounds within the alginate beads. In addition, bands corresponding to the skeletal stretching vibration of the aromatic rings and = C-O-C group of flavonoids (1076, 1516, and 1260 cm^{-1}) were also identified through some peaks also appear on the alginate, they appear with increasing intensity. The bands at 1430 cm^{-1} were assigned to C—N vibration. The distinct absorbance peak in the wavenumber region 3350 cm^{-1} can also be the result of the absorbance of water. The 1363 cm^{-1} absorption band may also be attributed to the O—H in-plane deformation in polyphe-

Table 5
Identified compound by GC–MS of JPE mixture extract (2:1, JE/PE).

Compound	Rt (min)	Molecular formula	CAS #	%A	Mass spectrum ions (m/z) [b]
Terpenes					
Monoterpenes					
(+)-α-Pinene	7.073	$C_{10}H_{16}$	7785-70-8	0.15	93, 91, 92, 77, 79(136)
(−)-β-Pinene	8.034	$C_{10}H_{16}$	18172-67-3	0.05	93, 91, 69, 77, 79(136)
3-Carene	8.661	$C_{10}H_{16}$	13466-78-9	0.20	93, 91, 79, 77, 92(136)
o-Cymene	8.978	$C_{10}H_{14}$	527-84-4	0.40	119, 91, **134**, 117, 115
(+)-Limonene	9.073	$C_{10}H_{16}$	5989-27-5	0.05	68, 93, 67, 79, 94(136)
Terpinen-4-ol	11.756	$C_{10}H_{18}O$	562-74-3	0.10	71, 93, 111, 136, **154**
α-Terpineol	11.976	$C_{10}H_{18}O$	98-55-5	0.10	59, 93, 121, 136, 81(154)
(−)-Cis-sabinol	12.109	$C_{10}H_{16}O$	3310-02-9	0.45	92, 79, 134, 81, 119(152)
Verbenone	12.183	$C_{10}H_{14}O$	1196-01-6	0.05	107, 91, 135, 80, **150**
Car-3-en-5-one	13.690	$C_{10}H_{14}O$	81800-50-2	0.77	**150**, 79, 135, 107, 91
Sobrerol	14.685	$C_{10}H_{18}O_2$	498-71-5	0.30	109, 59, 79, 137, 152(170)
Sesquiterpenes					
D-germacrene	16.075	$C_{15}H_{24}$	23986-74-5	0.25	161, 105, 91, 119, 81(204)
α-Muurolene	16.261	$C_{15}H_{24}$	10208-80-7	0.71	105, 161, 93, 119, 81(204)
γ-Cadinene	16.458	$C_{15}H_{24}$	39029-41-9	1.00	161, 105, 119, 91, 133(204)
δ-Cadinene	16.501	$C_{15}H_{24}$	483-76-1	2.67	161, 119, 105, 134, **204**
Elemol	16.868	$C_{15}H_{26}O$	639-99-6	0.77	93, 59, 107, 161, 81(222)
Spathulenol	17.261	$C_{15}H_{24}O$	6750-60-3	0.55	91, 105, 119, 159, 79(220)
(+)-Isospathulenol	17.842	$C_{15}H_{24}O$	88395-46-4	1.28	119, 91, 105, 159, 162(220)
τ-Cadinol	18.030	$C_{15}H_{26}O$	5937-11-1	2.53	161 105 119 121
τ-Muurolol	18.168	C15H26O	19912-62-0	4.35	95, 161 81(
α-Muurolene-14-ol	18.231	$C_{15}H_{24}O$	135118-51-3	0.85	159 117 105 81(
Ent-germacra-4(15),5,10(14)-trien-1β-ol	18.517	$C_{15}H_{24}O$	81968-62-9	1.71	109 79, 81(
6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro-naphthalen-2-ol	18.584	$C_{15}H_{24}O$	189102 [a]	1.56	159 131 105 **220**
Shyobunol	18.653	$C_{15}H_{26}O$	35727-45-8	0.82	84, 67, 105
Longifolenaldehyde	18.701	$C_{15}H_{24}O$	19890-84-7	0.87	95, 81, 121
Oplopanone	19.060	$C_{15}H_{26}O_2$	1911-78-0	1.23	135 153 111 107
Eudesma-4(14),7-dien-1β-ol	19.595	$C_{15}H_{24}O$	119120-23-9	0.90	159 107 119 79(
6-Epi-shyobunol	19.938	$C_{15}H_{26}O$	69350-61-4	4.60	81, 67, 55(
Platambin	20.688	$C_{15}H_{26}O_2$	58556-80-2	1.76	107 159 121 93(
Diterpenes					
Trans-geranylgeraniol	18.751	$C_{20}H_{34}O$	24034-73-9	1.21	69, 93, 121
Phytol	22.734	$C_{20}H_{40}O$	150-86-7	7.00	71, 123 95(
Alcohols					
2-Ethylhexanol	9.030	$C_8H_{18}O$	104-76-7	0.1	57, 70, 98(
Benzyl alcohol	9.138	C_7H_8O	100-51-6	0.24	79, 77,
2-Phenylethanol	10.585	$C_8H_{10}O$	60-12-8	0.40	91, **122** 57
Phenolic compounds					
Phenol	8.000	C_6H_6O	108-95-2	0.1	**94**, 65
3-Ethylphenol	11.425	$C_8H_{10}O$	620-17-7	0.36	107 **122** 79
Pyrogallol	14.388	$C_6H_6O_3$	87-66-1	8.79	**126** 108 51

Table 5 (*Continued*)

Compound	Rt (min)	Molecular formula	CAS #	%A	M sp ic z
4-Acetylphenol	15.358	$C_8H_8O_2$	99-93-4	0.60	1 1
Aspidinol	21.964	$C_{12}H_{16}O_4$	519-40-4	0.50	1 1 1 5
α-Tocopherol	27.108	$C_{29}H_{50}O_2$	59-02-9	2.44	1 4 2 1
Esters					
Triacetin	14.015	$C_9H_{14}O_6$	102-76-1	1.33	1 1 1 7
Isobutyl phthalate	20.301	$C_{16}H_{22}O_4$	84-69-5	1.44	1 1 2 1
Ethyl palmitate	21.611	$C_{18}H_{36}O_2$	628-97-7	0.91	8 1 1 2
Hexanedioic acid, bis(2-ethylhexyl) ester	25.156	$C_{22}H_{42}O_4$	103-23-1	1.20	1 1 5 1
2-(Dimethylamino)ethyl carbamate	25.693	$C_5H_{12}N_2O_2$	4220-32-0	0.50	5 7 5
Others					
Coumaran	12.245	C_8H_8O	496-16-2	2.24	1 1
1,6-Anhydro-β-D-glucopyranose	15.977	$C_6H_{10}O_5$	498-07-7	0.37	6 5 9
2(3H)-benzothiazolone	18.270	C_7H_5NOS	934-34-9	0.25	1 1 1
Linoleic acid	22.911	$C_{18}H_{32}O_2$	60-33-3	8.93	6 9 1
Not identified				29.8	

Rt: retention time; %A: relative compound area.

[a] Nist number.

[b] Ions listed in decreasing order of intensity; highlighted in bold the molecular ion when present among principle ions; and displayed in brackets (ion) in case the molecular ion is not present among principle ions.

Table 6
Total monomeric anthocyanin (TMAC) and total phenolic (TPC) contents.

Source	TMAC (mg/L)	TPC (mg GAE/L)
Jabuticaba (JE)	1318.0 ± 86.8	9.3 ± 0.8
Propolis (PE)	0.0 ± 0.0	6.8 ± 0.1
Jabuticaba + propolis mixture (JPE)	1105.8 ± 42.1	20.0 ± 0.7
JPE/alginate beads	991.7 ± 73.8	19.6 ± 0.7
Encapsulation efficiency (EE%)	89.6	98.1

nols. The deformation vibration of the carbon-carbon bonds in the phenolic groups adsorb in the region of 1500–1400 cm^{-1}.

3.5. Encapsulation efficiency EE (%)

The encapsulation efficiency was performed by adding the beads on a sodium citrate solution since it causes the destabilization of the calcium alginate matrix. It was believed that Ca^{+2} ions of the alginate network were supposed to exchange by Na^+ ions from the solution, weakening the gel structure. However, a change of anthocyanin extract colour from red to brown was observed indicating anthocyanin degradation, which was also observed by Santos et al. [45]. Thus, the EE (%) was determined based on the concentration of anthocyanin extract not encapsulated. For the total phenolic content, the same methodology was applied. The loss of anthocyanin extract to the gelling solution upon the crosslinking process was about 10.4 ± 3.4%, leading to EE (%) of 89.6%. The total phenolic achieved higher EE, about 98.1%. Hence, alginate has shown to be a compatible matrix for encapsulation biochemical active compounds extracted from natural. Other bioactive extracts encapsulated systems using the alginate dripping method also presented high EE(%): 99.3% for propolis [46] and 98.67% for jabuticaba extract [45] (Table 6).

3.6. The release profile of TMAC from JPE/alginate beads

Regardless of the anthocyanin health benefits, the rapid degradation after intake of a beverage rich in the bioactive limit its bioavailability in the plasma (<6% of initial dose). This behaviour is generally attributed to their low absorption occurred due to their low water solubility, poor stability, passive diffusion, and active efflux in the gastrointestinal tract [9]. The encapsulation of bioactive ingredients can improve its bioavailability during human digestion, by enhancing its solubility, protecting against the adverse condition in a certain part of the digestive tract (e.g. stomach) and delivering it to the target environment (e.g. intestine), resulting in a better absorption [47].

Since the main goal of this study is to develop JPE/alginate beads for use as a beverage additive, e.g. an oral route of administration; the target site for the extract release is the gastrointestinal environment. Therefore, the release profile of anthocyanins compounds was evaluated at gastrointestinal pH conditions. At gastric conditions, the pigment was released slower in the first 30 min with only about 7% of anthocyanin release. Upon 30 min the red colour, characteristic of anthocyanins, increased its intensity; and even after 240 min, the beads maintained its spherical shape integrity. The total amount of anthocyanins released from the beads was about 43.1% in the gastric simulated condition after 240 min. This restricted release (pH 1.2 in SGF) is attributed to the ion exchange phenomenon, in which the interchain calcium ions are replaced by H^+ occurring the carboxylic acid group protonation in the alginate chain [21,48]. This protonation results in the formation of insoluble alginic acid that limit water penetration into the particles and, helps to maintain its polymeric network. The slower swelling rate is a limiting factor for gastric release, thus, increasing the anthocyanins and phenolic compounds protection. This behaviour is in accordance with previous studies, reporting that the detainment of encapsulated substances under gastric conditions occurs by the shrinkage of the alginate hydrogel, under low pH medium [49]. Despite JPE/Alginate beads released 43.1% in the SGF, their colour remained, indicating that the anthocyanins were present in its active form, and were effectively protected by the alginate capsule until reaching the stomach environment. These results suggest that when orally consumed, the alginate beads will reach the stomach environment owning its antioxidant potential from the anthocyanins.

In the simulated intestinal phase (SIF), the release was faster. The integrity of the alginate beads was maintained in the first 30 min

Table 7
Kinetic analysis and release data of JPE/alginate beads.

Models		Zero-order	First-order	Higuchi	Hixon-Crowell	Korsmeyer-Peppas	n [a]	Release mechanism model
pH 1.2	R^2	0.9179	0.4752	0.9503	0.9339	0.9344	0.7369	Anomalous transport
	RMSE	4.0848	10.3262	3.1789	3.6640	0.2149		
	AIC_c	44.07	53.35	41.57	42.99	39.79		
pH 7.4	R^2	0.979	0.7124	0.9004	0.9821	0.9988	0.7395	Anomalous transport
	RMSE	4.6557	17.0867	10.0556	4.2680	0.0287		
	AIC_c	45.38	58.38	53.08	44.51	36.46		

[a] Release exponent (n) for the Korsmeyer-Peppas model, which best fitted to experimental data.

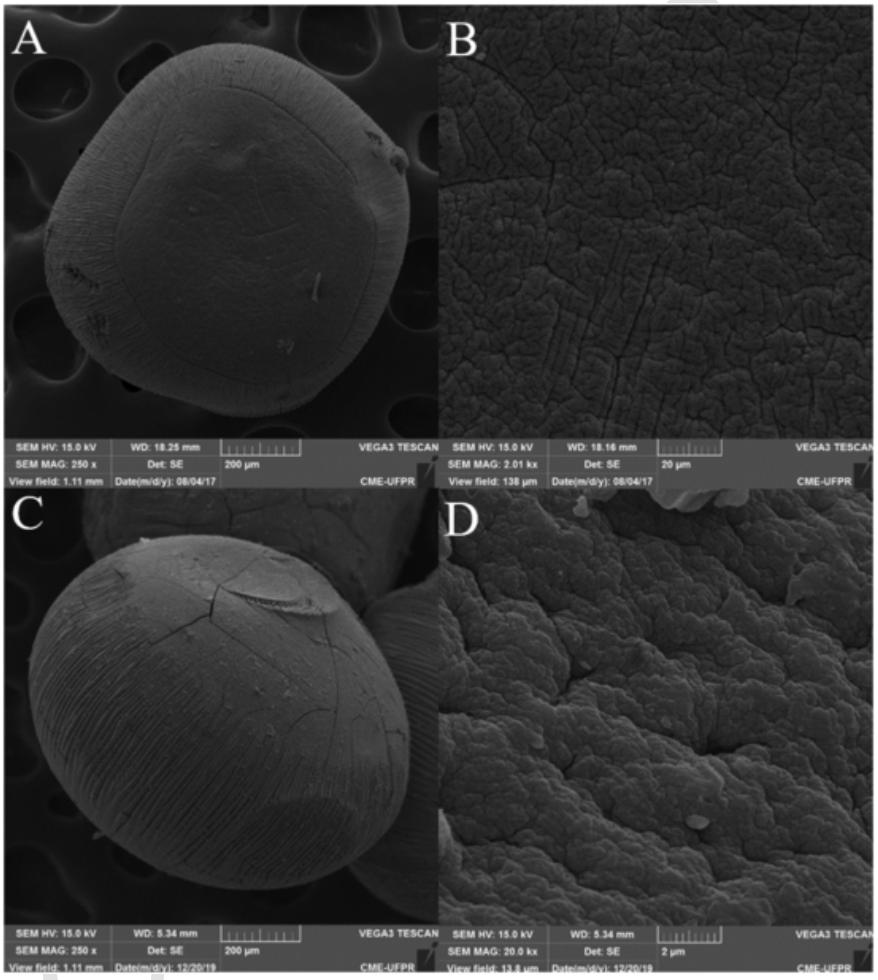

Fig. 3. SEM micrographs of plain alginate beads 250 × (A) and 20kx(B) and JPE/alginate beads 250 × (C) and 20kx(D).

and, after this period, they began to disintegrate due to pH 7.4 and the brown colour from the bioactive released, indicated the pigment degradation. The stability of anthocyanins highly dependent on the pH conditions which is unstable at neutral pH; also, the alginate beads, are unstable when immersed in a phosphate buffer (pH 7.4) and this is due to the ion exchange that results in the polymeric chain disintegration. The Ca^{2+} ions, that are linked to the –COO– units of polymannuronate (M blocks) sequence of alginate structure, are exchanged by the Na^+ from the saline solution. As a result, the electrostatic repulsion between the negative charges of the –COO– groups increase, causing chain relaxation and increased swelling of gel. The chain relaxation allows the interaction of the phosphate buffer ions with Ca^{2+}; thus, forming calcium phosphate. Furthermore, the Ca^{2+} ions that were linked to the –COO– units of polyguluronate (G blocks), also begins to be exchanged for the Na^+ ions of phosphate buffer. Since the polyguluronate sequences have strong importance on binding to calcium ions - forming the tight "egg-box" structure, the gel beads begin to disintegrate [50], releasing the JPE extract to the medium. Possibly, the complete release of the anthocyanin and total phenolic rich extract could be accomplished at 7.4 pH, but in the case of anthocyanins, this pH condition is destructive and could not provide the potential antioxidant activity that this bioactive could reach. Our results are in agreement with other reports, showing that phenolic compounds from propolis extract were released from algi-

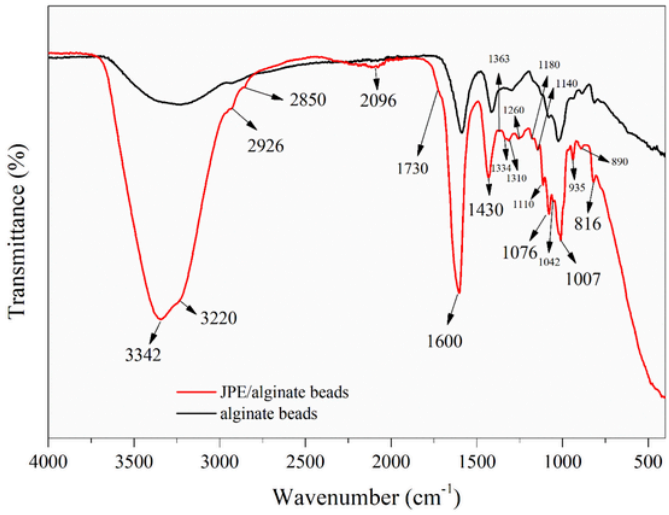

Fig. 4. FT-IR spectrum of plain alginate and JPE/alginate beads.

nate beads more effectively at pH 7.4 compared to pH 1.2 [46] (Fig. 5).

The JPE bioactive release from swelling polymeric matrix is influenced by several factors, including the extract and polymer characteristics as well as the release medium. The compound diffusion, polymer swelling, and degradation are usually the main driving forces in the release process from polymeric matrices. To determine the anthocyanins release kinetic mechanism from the JPE/alginate beads, the zero-order, first-order, Higuchi, Hixson-Crowell, and Korsmeyer-Peppas kinetic models were fitted to the experimental data. The best fit was evaluated using the coefficient of determination (R^2), the Root Mean Square Error (RMSE) and the corrected Akaike information criteria (AIC_c) (see Supplementary material B). The power-law or Korsmeyer-Peppas [51] is a simple and a semi-empirical model which better described the system (highest R^2, lowest RMSE and AIC), the model is given by Eq. (5):

$$\frac{M_t}{M_\infty} = k\, t^n \tag{5}$$

where M_t/M_∞ is the cumulative fraction of active released compound at time t, k is the release rate constant (dimension of time^{-1}), and n the value of the release exponent (dimensionless), which indicates whether the release mechanism correlates to a Fickian diffusion or non-Fickian diffusion model.

For gastrointestinal conditions, the obtained exponent value n for JPE/alginate beads was 0.7369 and 0.7395 (Table 7), indicating that the mass transfer follows a non-Fickian diffusion mechanism. Release exponents values $0.43 < n < 0.85$, for sphere geometry, indicate so-called "anomalous" transport, thus, occurring an overlapping of different types of phenomena, potentially including the bioactive diffusion and the polymer swelling.

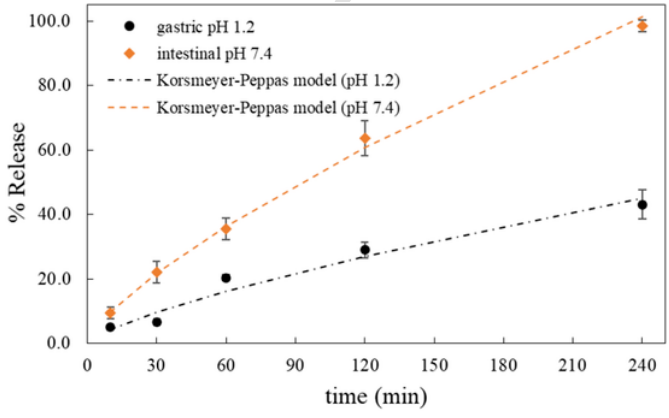

Fig. 5. Total monomeric anthocyanin % release.

According to Peppas [51], the dynamic swelling behaviour of hydrogels is dependent on the relative contribution of diffusion and polymer relaxation. In the ionic hydrogels, relaxation is affected by the ionization of functional groups, and an increase in the degree of ionization results in the electrostatic repulsion between ionized functional groups, leading to chain expansion, which in turn affects macromolecular chain relaxation. Thus, the swelling mechanism becomes more relaxation-controlled when the ionization of hydrogel increases. This release mechanism strongly supports the above-mentioned ionic exchange of alginate causing chain relaxation and beads disintegration under pH 7.4.

4. Conclusion

The Sabará jabuticaba fruit peel and Tubuna bee propolis, rich sources of phenolics and antioxidants compounds, had their extracts successfully encapsulated in alginate beads, and both pure extracts presented significant total phenolic content and high antioxidant activity by ABTS and DPPH assays. The pure jabuticaba extract also presented a high concentration of monomeric anthocyanin, which is an important source of pigment that can be useful for the beverage industry. The JPE mixture presented an enhancement of both total phenolic content and antioxidant activity. The GC–MS revealed a complex volatile profile for PE and JE extracts and corroborates with the antioxidant activity enhancement of JPE extract indicating a synergic interaction when compared to the extracts individually. The encapsulation efficiency achieved 89.6% for total phenolic and 98.1% for total monomeric anthocyanin concentration. The anthocyanin release mechanism followed a non-Fickian diffusion with a combination of diffusion and chain relaxation. As a result, this study demonstrates that anthocyanins and phenolic compounds encapsulation can be significantly sustained onto alginate hydrogel beads, suggesting their potential application as an oral delivery system, and enabling its usage as a natural colorant additive in food or beverages.

Author's contributions

Ithiara Dalponte Dallabona: Conceptualization, Data Curation, Investigation, Methodology, Resources, Formal analysis, , Writing – Original Draft and Review & Editing; **Gabriel Goetten de Lima**: Conceptualization, Writing – Original Draft and Review & Editing, Formal analysis, Investigation; **Beatriz Isabella Cestaro**: Methodology, Investigation, Writing – Original Draft; **Cristiane Vieira Helm**: Conceptualization; Resources, Supervision, Methodology, Project administration; **Regina Maria Matos Jorge**: Conceptualization; Supervision, Project administration, Writing – Original Draft and Review & Editing; **Alvaro Luiz Mathias**: Supervision, Writing – Original Draft and Review & Editing; **Ivisson de Souza Tasso**: Data Curation; **Thainnane Silva Paiva**: Data Curation; **Emanuele Joana Gbur Laureanti**: Data Curation; **Bruno José Gonçalves da Silva**: Resources, Review & Editing; **Luiz Mario de Matos Jorge**: Resources, Review & Editing. All authors provided critical feedback and helped to shape the research, analysis, and manuscript.

Declaration of competing interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijbiomac.2020.07.256.

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