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Microencapsulation of an anthocyanin-rich blackberry (*Rubus* spp.) by-product extract by freeze-drying

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ABSTRACT

One approach to improving sustainable food production is to add value to fruit by-products, which are currently used as animal feed or discarded, yet may be useful sources of natural antioxidants due to their phenolic compounds. Hence, the present work aimed to produce and evaluate two products prepared from an anthocyanin-rich extract of a blackberry by-product through freeze-drying. Maltodextrins with 10 and 20 dextrose equivalent (DE), were assessed as the carrier matrices. The maltodextrin DE did not significantly influence the mean diameter and solubility of the particles. Morphological analysis revealed that all the particles exhibited a broken glass structure and shriveled surfaces. Comparatively, better results were obtained from the maltodextrin 10 than 20DE powders, regarding anthocyanin retention in the drying process, hygroscopicity, moisture content, acidity, water activity and color indices ($P < 0.05$). The results suggest that blackberry by-products contain valuable biocompounds, namely anthocyanins. Therefore, the anthocyanin extraction, concentration and microencapsulation with maltodextrin 10DE, presented a potential approach to using blackberry by-products as food colorants or healthy ingredients.

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1. Introduction

Studies have shown that agricultural and industrial by-products from berries and other fruits and vegetables are good sources of bioactive compounds (Balasundram, Sundram, & Samman, 2006; Machado, Pasquel-Reátegui, Barbero, & Martínez, 2015) that could be used as nutraceuticals (Gorinstein et al., 2011). From an economical perspective, it is interesting to recover agro-industrial by-products as raw material for processing new food, pharmaceutical and cosmetics, which might enable a sustainable industrial production system, adding value to a food waste and reducing its disposal into the environment (Machado et al., 2015). Blackberries are increasing in economic value because of their high nutritional value and benefits to physical and mental health (Franceschinis, Salvatori, Sosa, & Schebor, 2014; Ivanovic et al., 2014), which can be largely attributed to their phenolic compounds, such as phenolic acids, tannins and anthocyanins (Branco et al., 2016; Kaume, Howard, & Devareddy, 2011).

Anthocyanins are water-soluble pigments, responsible for the red to purple shades of plant foods (Laokuldilok & Kanha, 2015). This group of phenolic compounds is associated with a high antioxidant activity, hence, the consumption of plant foods containing anthocyanins is linked to prevention of cardiovascular and neurological diseases, cancer and diabetes (Branco et al., 2016; Konczak & Zhang, 2004). Moreover, anthocyanins could be a good substitute for synthetic pigments. However, anthocyanins are unstable due to their sensitivity to temperature, pH, light and oxygen, for example (Patras, Brunton, O'Donnell, & Tiwari, 2010). Consequently, the food industry is constantly searching for practical and economical methods of conserving the color and bioactive properties of anthocyanins. Freeze-drying is considered a suitable method for drying heat-sensitive pigments. It is based on dehydration by sublimation of a frozen product and, during this procedure, the core materials and matrix solutions are homogenized and then co-lyophilized, resulting in a dry material (Laokuldilok & Kanha, 2015; Wilkowska, Ambroziak, Czyżowska, & Adamiec, 2016).

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Another technology which has been used to protect sensitive materials is encapsulation, which consists of packaging materials in the form of micro- or nanoparticles (Jafari, Assadpoor, He, & Bhandari, 2008). The choice of coating material is very important because it may influence the encapsulation efficiency and stability of the capsules (Wilkowska et al., 2016). Maltodextrin (MD) is the most commonly used encapsulating agent, due to its high water solubility, low viscosity and low sugar content (Bakowska-Barczak & Kolodziejczyk, 2011). MDs with a dextrose equivalent (DE) between 10 and 20 are widely used to encapsulate anthocyanins and phenolic acids (Ahmed, Akter, Lee, & Eun, 2010; Silva, Constant, Figueiredo, & Moura, 2010; Tonon, Brabet, & Hubinger, 2010).

In this context, the objective of the present work was to use freeze-drying and MD (10 and 20DE) as the carrier agent, to encapsulate a concentrated anthocyanin extract isolated from a blackberry (*Rubus* spp.) by-product and to evaluate the physicochemical properties of the obtained microparticles.

2. Material and methods

2.1. Anthocyanin extraction

Frozen blackberry pulp by-product (moisture 56.1% and 5.0% soluble solids) was provided by Ricaeli Company located in the city of Cabreúva, São Paulo, Brazil. It was stored in a freezing chamber at −10 °C and thawed in a refrigerator (7–8 °C) for 24 h.

Water was added at a 1:3 by-product-to-water (m/v) ratio, and the mixture shaken at room temperature in the dark for 8 h. The extract was then filtered through a sieve and concentrated using a rotatory evaporator at 60 °C, until 1/3 of the initial volume remained (Souza, Thomazini, Balieiro, & Fávaro-Trindade, 2015). The concentrated extract was stored in dark glass recipients in the fridge, until analysis.

2.2. Microencapsulation of anthocyanin extract by freeze-drying

MDs (10 and 20DE; Cargill Inc., Brazil) were respectively added to the concentrated extract to obtain 30% total solids concentration, followed by mechanical homogenization. The mixtures were then freeze-dried using a lyophilizer (Liotop L101, São Carlos, Brazil) at a constant temperature of −20 °C. The dried samples were ground using a mortar and pestle and the powders were packed in polyethylene bags and stored in the dark, until further analysis.

2.3. Characterization of the anthocyanins extract

The concentrated anthocyanin extract and the freeze-dried powders were characterized for their soluble solids (°Brix) by refractometry, pH using a pH meter, and acidity, based on titrimetric analysis with NaOH solution (0.1 N), as described by the Association of Official Analytical Chemists (AOAC, 2000).

2.4. Total anthocyanin content

The total anthocyanin content of the aqueous and concentrated extracts, as well as the lyophilized powders, was determined according to Fuleki and Francis (1968). The extraction was performed with 95% ethanol and 1.5 N HCl (85:15). Spectrophotometric absorbance (Biospectro Sp-220, Equipar, Curitiba, Brazil) was measured at 535 nm. The results were expressed as mg of cyanidin 3-glucoside/100 g of sample (dry basis). The retention of the anthocyanins after concentration and freeze-drying was also calculated.

2.5. Characterization of the microcapsules

2.5.1. Moisture content and water activity (a_w)

The moisture content of the powder was determined gravimetrically. Samples were weighed and dried in an oven, with air circulation, at 105 °C for 24 h (AOAC, 2000). The a_w was measured in an Aqualab CX-2 (Decagon, Pullman, USA) hygrometer, at 25 °C.

2.5.2. Hygroscopicity

The hygroscopicity of the powdered samples was assessed according to Tonon, Brabet, and Hubinger (2008), with modifications. Samples of each powder were stored at 20 °C, in desiccators containing saturated sodium chloride (NaCl) solutions (75% relative humidity; $a_w = 0.75$). The samples were weighed after 1 week, and the hygroscopicity was expressed as grams of absorbed moisture per 100 g of dry solids (g/100 g).

2.5.3. Solubility

Powder solubility was evaluated based on the method described by Cano-Chauca, Stringheta, Ramos, and Cal-Vidal (2005), with some modifications. The powder (0.5 g) was dissolved in 50 mL of distilled water and stirred at room temperature for 30 min. The suspension was then transferred to a tube and centrifuged at 3000 g for 5 min. An aliquot (25 mL) of the supernatant was transferred to a pre-weighed Petri dish and dried at 105 °C for 5 h. After drying, the dried weight of the soluble solid was measured and used to calculate the percentage solubility.

2.5.4. Color measurement

The color of the blackberry powder was measured using a colorimeter (HunterLab MiniScan EZ, Reston, USA), with a CIE Lab scale (L*, a* and b*). The color measurements were expressed in terms of lightness L* [0 (black) to 100 (white)] and the chromaticity parameters a* [green (−) to red (+)] and b* [blue (−) to yellow (+)]. The chroma (C*) or color intensity, and hue angle (H°) [0° (pure red color), 90° (pure yellow color), 180° (pure green color) and 270° (pure blue color)], were calculated by using Eqs. (1) and (2), respectively. The measurements were performed in triplicate and three readings were done for each replicate.

$$C* = \left[(a)^2 + (b)^2\right]^{1/2} \quad (1)$$

$$\text{Hue angle}(^\circ) = \tan^{-1}\left(\frac{b*}{a*}\right) \quad (2)$$

2.5.5. Particle morphology and size distribution

The microstructure of both freeze-dried powders was evaluated by scanning electron microscopy (SEM) (Topcon SM300, Tokyo, Japan), according to Tonon et al. (2008). The powders were attached to double-sided adhesive tape, fixed to SEM stubs and coated with gold/palladium, at a coating rate of 0.51 Å/s, 3–5 mA, 1 V and 2×10^{-2} Pa for 180 s. The SEM was operated at 5 kV with a magnification of 500 and 1000×. The particle size was determined using a laser light diffraction instrument (Shimadzu Sald-201V, Tokyo, Japan). A small amount of powder was suspended in isopropanol under magnetic agitation, and the particle size distribution was monitored until successive readings became constant. The particle size was expressed as D[4,3], also known as the De Brouckere mean diameter, which is the volume mean diameter and is generally used to characterize a particle.

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