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Encapsulating anthocyanins from *Hibiscus sabdariffa* L. calyces by ionic gelation: Pigment stability during storage of microparticles

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ABSTRACT

Hibiscus extract (HE) has a strong antioxidant activity and high anthocyanin content; it can be used as a natural pigment, also adding potential health benefits. The objective of this work was the microencapsulation of HE anthocyanin by ionic gelation (IG) using two techniques: dripping-extrusion and atomization, both by means of a double emulsion (HE/rapseed oil/pectin) and a cross-linked solution ($CaCl_2$). Particles (77–83% moisture content) were conditioned in acidified solution at 5, 15 and 25 °C, absence of light, and evaluated for anthocyanins and color for 50-days. The median diameter (D_{50}) of the particles ranged from 78 to 1100 μm and encapsulation efficiency ranged from 67.9 to 93.9%. The encapsulation caused higher temperature stability compared with the free extract. The half-life ($t_{1/2}$) values of the particles ranged from 7 (25 °C) to 180 days (5 °C) for anthocyanins and from 25 (25 °C) to 462 days (5 °C) for Chroma value. The IG increased the stability of HE anthocyanin. Both the dripping-extrusion and the atomization have shown to be feasible techniques.

1. Introduction

The use of plants as source of anthocyanins for phytonutrients and natural colorants has been studied by different authors (Mohd-Esa, Hern, Ismail, & Yee, 2010, Santos, Albarelli, Beppu, & Meireles, 2013; Da-Costa-Rocha, Bonnlaender, Sievers, Pischel, & Heinrich, 2014; Otálora, Carriazo, Iturriaga, Osorio, & Nazareno, 2016; Aizpurua-Olaizola et al., 2016).

Hibiscus (*Hibiscus sabdariffa* L.) is an herbaceous plant widely cultivated in tropical and subtropical areas of both hemispheres (Sinela et al., 2017). Its calyx can be used in the preparation of a slightly astringent and acid aqueous infusion. Geographical regions with tropical climate such as Sudan, Thailand, China, Mexico, Egypt, Senegal, and Tanzania are among the main commercial producers of this plant (Domínguez-Lopez, Remondetto, & Navarro-Galindo, 2008).

The hibiscus extract has antibacterial and antioxidant activity as well as hepatoprotective action, alleviating hunger and having effects on lipid metabolism (anti-cholesterol). It also has diuretic, anti-diabetic, anti-hypertensive effects, anti-inflammatory activities and other biological effects, such as cancer prevention and liver protection activities (Zhen et al., 2016). The presence of phenolic acids (especially protocatechuic acid), organic acids (hydroxycitric acid) and anthocyanins (delphinidin-3-sambubioside and cyanidin-3-sambubioside) may also contribute to the effects reported (Da-Costa-Rocha et al., 2014).

The ingestion of natural bioactive compounds, such as polyphenols and anthocyanins is of great interest, but the difficulties associated with the susceptibility of these compounds to adverse external effects, or damaging conditions of food processing and their chemical instability, have provided much effort to improve oral bioavailability. Therefore, microencapsulation represents a promising concept. The usage of microencapsulated bioactive compounds as functional ingredients in various food and beverage applications presents significant potential, as it may allow the enrichment of various food products with natural antioxidants (Diplock et al., 1999). Such ingredients are mainly wrapped in a wall material, thereby giving useful and/or eliminating useless properties of the original ingredient (Gharsallaoui, Roudaut, Chambin, Voilley, & Saurel, 2007).

In addition to being related to health benefits, such as antioxidants and anticancer agents, anthocyanins can be used as colorants. It is a water-soluble pigment and an alternative to the use of red to blue colors

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in foods (Reynertson et al., 2006). The replacement of artificial by natural colorants has gained consumer attention due to concerns about toxic and carcinogenic effects of artificial colorants and the growing search for healthy foods (Ko, Lee, Nam, & Lee, 2017). However, the use of anthocyanins is impaired by the instability of these molecules to adverse processing conditions and food storage. One of the ways of effectively protecting the anthocyanin compounds in the processed product is the microencapsulation.

Ionic gelation is a microencapsulation method that can be conducted by using atomization, dripping (coextrusion, extrusion), or electrostatic spray procedures. This method has the advantage of using mild conditions, since it does not employ high temperatures, vigorous stirring or organic solvents, enabling encapsulation of substances that would degrade under other conditions (Colak et al., 2016; Mukai-Corrêa, Prata, Alvim, & Grosso, 2005). A disadvantage is that encapsulation of hydrophilic or low molecular materials showed problems of easy diffusion and fast release through the ionic gel network regardless of pH (Kim, Lee, & Lee, 2016). Some strategies need to be applied (i.e. emulsion system, coating material) to retain the hydrophilic active compounds, since the ionic gelation has direct applicability for hydrophobic or low soluble active compounds only (Oehme, Valotis, Krammer, Zimmermann, & Schreier, 2011, Henning, Leick, Kott, Rehage, & Suter, 2012). The emulsion gelation technique using oil has been reported to create a barrier against the loss of hydrophilic compounds (Kim et al., 2016).

The ionic gelation technique for anthocyanin encapsulation was successfully used by several authors (Belscak-Cvitanovic et al., 2016; Santos et al., 2013; Yamdech, Aramwit, & Kanopanont, 2012). The unfavorable points shown by the authors when using this technique are the larger size and low stability of particles (mainly for hydrophilic active compounds). The favorable points are low polydispersity and good encapsulation efficiency. Macro and microparticles have been investigated in separate independent studies with different preparation conditions and core materials. Therefore, assessing the macro and microparticles with coherent conditions in a single study can reveal the characteristics of particles more clearly (Kim et al., 2016).

This work had the purpose of encapsulating the anthocyanin extract from hibiscus calyces by using a double emulsion and two ionic gelation methods (dripping – extrusion and atomization), as well as evaluating the stability of microcapsules at different storage temperatures.

2. Material and methods

2.1. Material

The following were used: food grade (30 g/100 g) hibiscus extract (*Hibiscus sabdariffa* L.) provided by *Heide Extratos Vegetais company*, Pinhais/PR; GENU® amidated low methoxyl pectin (CP Kelco, Limeira/SP); food grade calcium chloride (*Dinâmica*, Diadema/SP); rapeseed oil: commercial brand Liza oil (Cargill Agrícola S.A., Mairinque/SP); analytical grade reagents – PA and PGPR (polyglycerol polyricinoleate) surfactant (*Danisco Brasil Ltda*, Brazil).

2.2. Encapsulation of anthocyanin extract

In order to obtain hydrosoluble extract retention in gel matrix (also rich in water), a simple emulsion (w/o) was produced at first with rapeseed oil and PGPR surfactant (4 g/100 g) at a ratio of 35:65 w/w in ultra-turrax IKA T18 (15,000 rpm/15 min) disperser under controlled temperature (25 °C). Then a double emulsion (w/o/w) was produced with a pectin solution (2 g/100 g) in ultra-turrax IKA T18 (15,000 rpm/5 min) at a 20:80 w/w ratio.

2.2.1. Encapsulation by using dripping – extrusion method

Particles were produced using the Encapsulator equipment (Büchi B-390), nozzle diameter of 300 μm and air pressure of 200 mbar. The cross-linking solution was $CaCl_2$ (3 g/100 g). The following variables were considered on particle production: vibration frequency: 100–2200 Hz; electrode tension: 400–2000 V. The applied feed rate was 11.5 ml/min. The distance between the atomizer and the surface of $CaCl_2$ solution was 10 cm. The microspheres were stirred at 100 rpm for 15 min for hardening.

2.2.2. Encapsulation by atomization method

The particles were formatted by spraying, applying a double-fluid atomizer (mini spray dryer B-290 – nozzle 0.7 mm, adapted outside equipment), where double emulsion was sprayed on the cross-linking solution ($CaCl_2$ 3 g/100 g). For the experiments, the following variables were considered: air pressures ranging from 0.15 to 0.23 bar and feeding rates ranging from 0.70 to 1.61 ml/min. The distance between the atomizer and the surface of $CaCl_2$ solution was 18 cm. The microspheres were stirred at 100 rpm for 15 min for hardening.

2.3. Microparticles characterization

2.3.1. Mean diameter and size distribution

The samples had their mean diameters measured and size distribution determined in Laser Diffraction Analyzer LA-950V2 (Horiba Instruments, Inc., Japan) by using the liquid dispersion module with filtered water as dispersion medium. The mean diameter was expressed in two ways: based on the mean diameter of a sphere with the same volume (De Brouckere diameter – $D_{[4,3]}$), defined by Eq. (1), and median value (defined by the diameter that divides the population into two equal parts of 50% of accumulated volume) called D_{50}

$$D_{[4,3]} = \frac{\sum n_i d_i^3}{\sum n_i d_i^2} \quad (1)$$

where d_i is the diameter of particles and n_i is the number of particles.

The polydispersity index (PDI) was calculated according to Jafari, He, and Bhandari (2007), by using Eq. (2):

$$PDI = \frac{d_{90} - d_{10}}{d_{50}} \quad (2)$$

where d_{10}, d_{50} and d_{90} are the diameters at 10%, 50% and 90% of accumulated volume, respectively.

2.3.2. Color measurement

Colorimetric determinations were conducted by using Chromameter CR-400 (Konica-Minolta Sensing Inc., Osaka, Japan), programmed in CieLab system. The reading of color of particles was performed after the samples were filtrated in filter paper and put in Petri dishes. The readings were made in quadruplicate. Equipment was calibrated with the white calibration plate before any reading.

Chroma and hue values were also calculated by Eqs. (3) and (4).

$$Chroma = C^* = \sqrt{(a*^2 + b*^2)} \quad (3)$$

$$Hue = H^* = arctan\left(\frac{b*}{a*}\right) \quad (4)$$

2.3.3. Morphology

Particles morphology was analyzed according to the methodology adapted from Alvim, Souza, Koury, Jurt, and Dantas (2013), using an optical microscope (model BX41, brand Olympus) with 40X and 100X magnifications.

2.3.4. Moisture content

The moisture content was gravimetrically determined by drying at 70 °C with no vacuum for 24 h, followed by vacuum drying for additional 24 h. The analysis was made in triplicate, and approximately 10 g of the sample were weighed (AOAC., 2006).