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Functional properties of encapsulated Cagaita (*Eugenia dysenterica* DC.) fruit extract

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ABSTRACT

Cagaita (*Eugenia dysenterica* DC.) is an important native Brazilian fruit species with potential health effects associated with its concentration and profile of polyphenols. The aim of this work was to evaluate the total phenolic (TPC), total proanthocyanidins, the antioxidant, antimicrobial, and anti-obesity potential of cagaita extracted, dried and encapsulated powders. Samples were produced with gum arabic or inulin as the carrier using different spray-drying conditions. *E. dysenterica* powders had a content of total phenolics between 9.9 and 31.2 mg GAE/g DW, which correlated with antioxidant capacity. Powders also showed inhibitory activity against *Staphylococcus aureus* and *Listerya monocytogenes* similar to vancomycin, erythromycin and ceftriaxone used as controls, and also showed inhibition of α-amylase and α-glucosidase. Preparation of dry extracts from cagaita using spray-drying is an alternative to prevent deterioration of the bioactive compounds present in the fruit, ensuring quality and availability of the fruit during the off-season.

1. Introduction

Brazil is the country with the highest biodiversity in the world and is the third largest producer of fruits (FAO, 2013). There are various native fruits with interesting potential as a source of polyphenols for the food and pharmaceutical industries. Cagaita or "cagaiteira" (*Eugenia dysenterica* DC.) is a native fruit belonging to the family of Myrtaceae and is native to the Cerrado, a Brazilian savannah biome. The whole fruit is consumed *in natura* and is an important raw material for the preparation of jam, juices and ice cream by small and middle-sized companies in Central Brazil (Rodrigues, Collevatti, Chaves, Moreira, & Telles, 2016). Despite the alleged medicinal properties of fruits (Donado-Pestana, Belchior, & Genovese, 2015; Lima et al., 2010), its use is limited due to the high perishability (between 5 and 6 days at ambient) and high transport costs.

Fruit of *E. dysenterica* are considered a good source of polyphenols, mainly flavonoids (Genovese, Pinto, Gonçalves, & Lajolo, 2008). Different activities have been associated with the presence of polyphenolic compounds in foods, such as antioxidant (Zhang et al., 2015), antimicrobial (Kolodziejczyk, Abadias, Viñas, Guyot, & Baron, 2013), and anti-obesity due to inhibition of key enzymes in the metabolism of carbohydrates such as α-glucosidase and α-amylase, as well as, the attenuation of fasting hyperglycemia, hypertriglyceridemia, and hypercholesterolemia (Donado-Pestana et al., 2015; De Souza Schmidt, Lajolo, & Genovese, 2010). Thus, the presence of phenolic compounds in cagaita may have therapeutic potential although these compounds are susceptible to degradation caused by factors such as pH, temperature, exposure to light, oxygen, and storage conditions (Grace et al., 2014; Selcuk & Erkan, 2015).

As a consequence of the limited use of the cagaita fruit and the susceptibility of the phenolic compound to degradation. The production of extract powders is an alternative to extend the shelf life of the product. One of the techniques use for this purpose is microencapsulation, in which the active agent (such as polyphenols) is entrap within another substance (wall material) (Mahdavi, Jafari, Assadpoor, & Dehnad, 2016). Different methods exist for the microencapsulation of active agents in the food industry. However, spray-drying is the most commonly used method, because it is a continuous, low cost process that produces dry particles of good quality, and for which the apparatus required is readily available (Mahdavi, Jafari,

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Assadpoor, & Ghorbani, 2016).

Spray-drying has been successfully used for encapsulation of different components that are susceptible to degradation (Esfanjani & Jafari, 2016; Mahdavi, Jafari, Ghorbani, & Assadpoor, 2014; Souza et al., 2014). The selection of the drying additives depends on the desired properties and the final application of the product. Gum arabic is one of the most common material used for microencapsulation due to its high solubility, low viscosity and film forming properties (Sarkar, Gupta, Variyar, Sharma, & Singhal, 2013). However, limited information is available on the use of inulin as a carrier. Both compounds are not hydrolyzed in the stomach and the small intestine (Ali, Ziada, & Blunden, 2009; Mensink, Frijlink, van der Voort Maarschalk, & Hinrichs, 2015), being fermented in the large intestine by microflora, which avoids the increase in the glycemic index, and makes them potential ingredients for foods for diabetics.

The aim of this study was to evaluated spray-drying of cagaita extracts using gum arabic and inulin as a carriers in relation to functional properties (TPC, *in vitro* antioxidant capacity, antimicrobial activity and the capacity to inhibit the key enzymes of carbohydrates metabolism).

2. Materials and methods

2.1. Materials

Frozen commercial pulp (fruit without seeds; stored at −20 °C and processed in September of 2013) of cagaita (*Eugenia dysenterica* DC.) was purchased in January of 2014 from Cooperativa dos Agricultores Familiares Agroextrativistas Grande Sertão (Montes Claros, MG, Brazil). The carriers used were food grade inulin extracted from chicory (SIBA ingredients Com. Ltd, São Paulo, SP, Brazil) and gum arabic (Nexira Brazil Com. Ltd., São Paulo, SP, Brazil). All reagents were of analytical grade.

2.2. Preparation and encapsulation of cagaita extract

The extract of cagaita pulp was prepared using aqueous ethanol (79%, v/v) at a ratio of 1:20 (w/v) for 3 min at 14000 rpm using a homogenizer (Ultra-Turrax T25; IKA, Staufen, Germany). The extract was then separated from the sample residue by centrifugation (5600*g* for 10 min; Centrifuge, Z446-K; Wehingen, Germany). Extraction of residue was repeated twice using the same conditions. The resultant extracts were pooled and concentrated at 40 °C in a rotatory evaporator (B-210; Buchi, Flawil, Sweden) until dryness. After that, the extract was mixed with different concentrations (10%, 20% and 30% w/v) of the carrier (inulin or gum arabic), and then spray-dried (model MSD 1.0, Labmaq; Sao Paulo, SP, Brazil) at different temperatures (120, 140 and 160 °C) using a peristaltic pump at a fixed rate of 0.60 m^3/min. The formulation was mixed continuously using a magnetic stirrer (Daza et al., 2016).

2.3. Freeze-dried sample

A control sample was obtained by lyophilization of the extract in a Pironi 501 freeze-dryer (Thermo Electron Corporation, New York, NY, USA) at −80 °C and 100 mTorr for 96 h.

2.4. Phenolic compounds and antioxidant capacity

2.4.1. Sample extraction

The spray-dried powders (0.5g) were extracted with 70% of aqueous methanol (20 mL) using the homogenizer for 3 min at 8000 rpm. The homogenized extract was centrifuged for 10 min at 5600*g* and the supernatant was concentrated at 40 °C in a rotatory evaporator and kept at −18 °C until subsequent analysis (no more than 3 days). All extractions were done in duplicate using the same batch of material, and the subsequent assays were run in triplicate.

2.4.2. Total phenolics content (TPC)

The determination was carried out according to Singleton et al. (1999), with some modifications. A 0.25 mL aliquot of the extract was mixed with 0.25 mL of the Folin-Ciocalteu reagent (Sigma Aldrich, St. Louis, MO, USA) and 2 mL of distilled water. After 3 min at room temperature (25 °C) sodium carbonate (Na_2CO_3, 0.25 mL) solution was added, and the mixture placed in a water bath for 30 min at 37 °C. The absorbance was read at 750 nm in a U1100 UV/Visible spectrophotometer (Hitachi Ltd., Tokyo, Japan). A calibration curve was prepared using gallic acid (Sigma Aldrich) at different concentrations (0 – 40 μg/mL). The results were expressed as mg of gallic acid equivalents per gram of sample on a dry weight basis (mg GAE/g DW). Retention of phenolic compounds was calculated with respect to the content of phenolic compounds of the extract before spray-drying.

2.4.3. Total proanthocyanidins content

The total content of proanthocyanidins was determined according to Porter, Hrstich, and Chan (1985), using a reagent comprised of 154 mg of $FeSO_4{\cdot}7H_2O$/L of *n*-butanol: hydrochloric acid (3:2). A total of 250 μL of each sample extract and 2.5 mL of the reagent were incubated at 90 °C for 15 min. The blank consisted of 2.5 mL of the reagent and 250 μL of methanol: acetic acid (99.5:0.5). The absorbance was measured at 540 nm using the spectrophotometer. A standard curve was prepared using quebracho tannin (QT Unitan, Buenos Aires, Argentina) at different concentrations (0 – 2.4 mg/mL). Results were expressed as mg of quebracho tannin/g DW. Retention was calculated with respect to the content of proanthocyanidins in the extract before spray-drying.

2.4.4. HPLC analysis of flavonoids

Extraction was done according to Arabbi, Genovese, and Lajolo (2004). The identification and quantification of the main flavonoids were done using a Prodigy ODS3 reversed phase silica column (5 μm, 250 × 4.6 mm, Phenomenex Ltd, Torrance, CA, USA), in an analytical reversed-phase HPLC HewlettPackard 1100 system (automatic sample injector, quaternary pump and diode array detector (DAD)) controlled by the ChemStation software (Hewlett-Packard, Palo Alto, CA, USA) as describe previously by Arabbi et al. (2004). Eluates were monitored at 270, 370, and 525 nm. The samples were injected in triplicate and the phenolic compounds were identified by comparing the retention and spectra time with appropriate standards (Quercetin ≥95% HPLC grade, Sigma Aldrich).

2.4.5. DPPH radical-scavenging ability

The antioxidant capacity was determined using the DPPH• (2,2-diphenyl-1-picrylhydrazyl) radical-scavenging method according to Brand-Williams, Cuvelier, and Berset (1995); with some modifications. An aliquot (50 μL) of the extract, previously diluted, was added to 250 μL of a methanolic solution of DPPH• (0.5 mM) and shaken. After 25 min at 25 °C the absorbance was measured at 517 nm using a microplate spectrophotometer (Synergy H1 hybrid reader, Bioteck, Chicago, IL, USA). A calibration curve was prepared using Trolox (Sigma Aldrich) at different concentrations (20 – 80 μM). Results were expressed as μmol of Trolox equivalent per gram of sample on a dry weight basis (TE/g DW).

2.4.6. Ferric reducing antioxidant power (FRAP)

The analysis was done according to Benzie and Strain (1996), with slight modifications. Twenty μL of previously diluted extract and 150 μL of the FRAP reagent were added and then incubated for 4 min at 37 °C. The absorbance was measured at 593 nm using the microplate spectrophotometer. An analytical curve was prepared using different concentrations of Trolox (0.1, 0.2, 0.4, 0.6, and 0.8 mmol/L) and FRAP data were expressed as mmol Trolox equivalents/g DW.