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Nanoencapsulation of passion fruit by-products extracts for enhanced antimicrobial activity

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ABSTRACT

Large amounts of passion fruit residues are underutilized by juice industries that can potentially be a source of bioactive compounds including antimicrobials. Co-precipitation with biodegradable polymers, including poly (DL-lactide-co-glycolide) (PLGA), may be used to enhance these compounds bioactivities and provide controlled release. This study aimed to produce PLGA particles of passion fruit by-products (seed and cake) extracts with different PLGA lactide to glycolide (50:50 and 65:35) ratios using the emulsion/solvent evaporation method (ESE). Characterization analyses indicated extracts encapsulation, controlled release and spherical shape for most treatments. Particle sizes ranged from 355 to 470 nm and entrapment efficiencies (EE) from 23.8 to 79%. The Gompertz model for bacterial growth fitted well the extracts release data. Results suggest that PLGA 65:35 is more suitable to both extracts encapsulation. Although, for the cake extract, EE was significantly lower than the seed extract. The ESE encapsulation using both PLGAs notably enhanced both extracts antimicrobial activities.

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1. Introduction

Brazil is the world's largest producer and consumer of fresh and processed yellow passion fruit (*Passiflora edulis* f. *flavicarpa*), accounting for 50–60% of the total world production, which was estimated to be in 1.4 metric tons in 2013 (USAID, 2014). The production of concentrated juice has the highest economic impact for the yellow passion fruit market as its demand is growing worldwide (USAID, 2014, 2011; von der Linden, 2007). The great volume of juice produced generates a large amount of by-products such as seeds and rind, but few studies can be found in the literature concerning the reuse of seeds (Ferreira et al., 2011; Malacrida and Jorge, 2012; Piombo et al., 2006). Seeds represent 4–12% of passion fruits composition and contain around 30% oil (Malacrida and Jorge, 2012), and great part of this by-product is still wasted. Part of it has been used to produce seed oil by conventional mechanical press finding var-

ious applications in the food, pharmaceutical and cosmetic industries, due to its high content of unsaturated fatty acids, especially linoleic acid (up to 70%) (Ferreira et al., 2011; Malacrida and Jorge, 2012; Piombo et al., 2006). The seed cake, a by-product derived from the cold press of the seeds from oil production, can be a potential source of bioactive compounds (anthocyanins, flavonoids, carotenoids, and phenolics) including antioxidants, antimicrobials and antitumor compounds as they are originally present in the seeds (Ferreira et al., 2011; Malacrida and Jorge, 2012; Sano et al., 2011; Silva et al., 2014a,b).

In a previous study, we demonstrated the antioxidant and antimicrobial activities of passion fruit seed and seed cake extracts obtained by different methods and solvents (Oliveira et al., 2016). Natural antioxidants and antimicrobials are an excellent way to improve food quality and shelf-life without introducing undesirable chemical preservatives. These materials can also be useful for cosmetics and pharmaceutical products; however, exposure to oxygen, heat and light can cause

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loss or reduction of extracts's bioactivities. Thus, co-precipitation with polymers may be used to preserve active compounds.

Co-precipitation or encapsulation with polymer have been largely applied to protect sensitive substances from adverse effects of the surrounding environment, mask odor or taste, control and target the delivery of active compound, improve aqueous solubility, among others. The polymer used plays an important role on the final product quality as, besides the protection, it determines how the encapsulated compounds will be released (Weiss et al., 2006). Poly(DL-lactide-co-glycolide) (PLGA) is a biocompatible and biodegradable polymer, FDA approved, that degrades by hydrolysis of the ester backbone into the homopolymers of lactic acid and glycolic acid, known products of cellular intermediary metabolism, meaning non-harmful and non-toxic compounds (Anderson and Shive, 1997; Astete and Sabliov, 2006; Park, 1995; Stevanović and Uskoković, 2009). Its application could be useful in the food industry to disperse hydrophobic compounds in hydrophilic compositions (Hill et al., 2013).

Extensive research led to a full range of PLGA polymers currently commercially available. The ratio of monomers glycolide to lactide at different compositions determines the degree of crystallinity and the rate of degradation of the polymers. The polymerization of crystalline PGA with PLA reduces the degree of crystallinity leading to increases in rates of hydration and hydrolysis. In general, the higher the content of glycolide, the quicker the rate of degradation (Cohn and Younes, 1987; Park, 1995).

Considering the potential bioactivity of the passion fruit by-products extracts and their potential applications in food and pharmaceuticals, the aim of this study was to produce PLGA particles of passion fruit by-products (seed and cake) extracts with two different lactide to glycolide (50:50 and 65:35) ratios of PLGA composition using the emulsion/solvent evaporation method (ESE) and evaluate their physico-chemical properties (size, morphology, entrapment efficiency, *in vitro* release) and their antimicrobial activities.

2. Methods

2.1. Raw materials and reagents

The raw materials were supplied by the company Extrair Óleos Naturais located in the State of Rio de Janeiro, Brazil. This company collects and processes the by-products from passion fruit juice producers. Cleaning (process under patent registration) and drying (60 °C in rotational drier) are performed on the same day. The dried seeds are used by the company to produce passion fruit seed oil by cold pressing. After the oil production, the remaining by-product is a brown dry powder called seed cake.

Seeds and seed cake were shipped to Federal University of Santa Catarina, SC, Brazil, where they were stored at –18 °C until their use. Upon arrival, the passion fruit seeds and seed cake presented a moisture content of $8.50 \pm 0.09\%$ (w/w) and $6.26 \pm 0.06\%$ (w/w), respectively, determined according to AOAC method 940.26 (AOAC International, 2005). Extracts were selected from a previous study of Oliveira et al. (2016), aiming to have one sample of each raw material that showed a good combination of extraction yield and biological activity, mainly antimicrobial. Briefly, the seeds were ground in a domestic blender (Black & Decker, SP, Brazil) prior to extraction with supercritical carbon dioxide (SC-CO₂) at 150 bar, 40 °C and constant solvent flow rate of 0.5 kg CO₂/h. While the seed cake did not require any pre-treatment to be macerated with a mixture of ethanol and water (1/1, v/v) (EtOH-H₂O).

PLGA, with copolymer ratios of DL-lactide to glycolide of 50:50 (Mw 30,000–38,000 g/mol, Resomer RG 503 ester terminated, Evonik, Essen, Germany) and 65:35 (Mw 40,000–75,000 g/mol, Sigma-Aldrich, St. Louis, MO) were used

in this study. The surfactant used in the emulsification process was polyvinyl alcohol (PVA) (98%–99% hydrolysis degree and average Mw 30,000–50,000 g/mol, Sigma-Aldrich). Dichloromethane, 99% acetonitrile (HPLC grade), D(+)-trehalose 98%, tryptic soy broth (TSB), tryptic soy agar (TSA), and peptone water were obtained from VWR International (West Chester, PA). All other reagents were of analytical grade and were used as obtained from the supplier without further processing.

2.2. Particle synthesis

Particles were produced using the emulsion solvent evaporation (ESE) method similar to the method outlined by Gomes et al. (2011). First, the organic phase was prepared by dissolving 50 mg of PLGA into 2 mL dichloromethane along with 16% (w/w relative to PLGA) of each extract. The organic phase was added drop-wise to 20 mL of aqueous 0.5% (w/w) PVA solution in 0.2- μ m filtered (Nalgene Filtration Products) water. This mixture was emulsified for 2 min at 9500 rpm using an Ultra-Turrax T25 basic Ika (Works, Inc., Wilmington, NC). The emulsion was sonicated in an ice bath for 30 min at 70 W (Cole Parmer sonicator 8890, Vernon Hill, IL), before removing the dichloromethane using a rotoevaporator (Buchi R-210 Rotavapor, Buchi Co., New Castle, DE) under vacuum (0.97 psi). Unloaded (control) particles were produced by the same method without adding extract in the organic phase. Dichloromethane was selected to prepare the particles since it is the most volatile solvent among the ones that solubilize PLGA, and it is a commonly used solvent for PLGA nanoencapsulation process (Astete and Sabliov, 2006). Then, the particles were purified by ultrafiltration to remove excess PVA and non-encapsulated extracts using a Millipore-labscale™ TFF system fitted with a 50 g/mol molecular weight cutoff Pellicon XL-Millipore (Millipore Co., Kankakee, IL). The particles were ultrafiltered with 200 mL of water, inlet pressure of 30 psi and outlet pressure of 10 psi, collecting the retentate when it reached 50 mL. After ultrafiltration, D(+)-trehalose was added to the particle solution at a 1:1 ratio relative to the PLGA to act as a cryoprotectant. The particles solution was kept at –20 °C until being lyophilized at –50 °C and 9.67×10^{-5} psi vacuum in a Labconco Freeze Dry-5 unit (Labconco, Kansas City, MO). Dried particles were collected and stored in a desiccator at –20 °C for further analysis. It is relevant to consider dichloromethane residue in the particles after their synthesis prior to any approval for food or pharmaceutical applications. Furthermore, most of the dichloromethane should be removed during the rotoevaporation step, and even if some dichloromethane traces would remain, they should be removed on the subsequent steps including ultrafiltration and freeze drying.

2.3. Particle size analysis and morphology

Particle size measurements were obtained by dynamic light scattering (DLS) in a Nano-ZS90 ZetaSizer (633 nm He-Ne 200, Malvern, UK). Particles were dispersed in 0.2 μ m-filtered distilled water at a concentration of 1 mg/mL and agitated until solubilized before analysis using 1-cm path length disposable polystyrene cuvettes at scattering angle of 90°, refractive index of 1.590 and temperature of 25 °C.

Aqueous suspensions of particles were examined using a FEI Morgagni Transmission Electron Microscope (TEM) (FEI Co., Hillsboro, OR) at the School of Veterinary Medicine and

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