Food Hydrocolloids 77 (2018) 297–306

Contents lists available at ScienceDirect

Food Hydrocolloids

journal homepage: www.elsevier.com/locate/foodhyd

Functional properties and encapsulation of a proanthocyanidin-rich cinnamon extract (*Cinnamomum zeylanicum*) by complex coacervation using gelatin and different polysaccharides

Volnei Brito de Souza [a, b, *], Marcelo Thomazini [b], Mariana A. Echalar Barrientos [b], Camila Marina Nalin [b], Roselayne Ferro-Furtado [d], Maria Inés Genovese [c], Carmen Sílvia Favaro-Trindade [b]

[a] Universidade Federal do Oeste da Bahia, Rua Professor José Seabra de Lemos – lado par, Recanto dos Pássaros, CEP 47808-021 Barreiras, Bahia, Brazil
[b] Universidade de São Paulo (USP), Faculdade de Zootecnia e Engenharia de Alimentos (FZEA), Avenida Duque de Caxias Norte, 225, CEP 13635-900, Pirassununga, São Paulo, Brazil
[c] Universidade de São Paulo (USP), Faculdade de Ciências Farmacêuticas (FCF), Departamento de Alimentos e Nutrição Experimental, Av. Prof. Lineu Prestes, 580 e Bloco 14, Caixa-Postal 66083, Butantã, CEP 05508-900 São Paulo, Brazil
[d] Embrapa Agroindústria Tropical, Rua Dra. Sara Mesquita 2270, CEP 60511-110, Fortaleza, CE, Brazil

ARTICLE INFO

Article history:
Received 16 May 2017
Received in revised form
12 September 2017
Accepted 27 September 2017
Available online 7 October 2017

Keywords:
Proanthocyanidins
Antioxidant capacity
Anti-diabetes activity
Microparticles
Proteins
Carbohydrates

ABSTRACT

The phenolic compounds present in the cinnamon extract are responsible for its beneficial effect on health. However, these compounds can undergo degradation reactions and have undesirable sensorial characteristics, which makes it difficult to apply and consume a large quantity of this extract. Thus, the present work evaluated cinnamon extract rich in proanthocyanidins in liquid, spray-dried and lyophilized forms for content of phenolic compounds, antioxidant and antimicrobial capacity, as well as inhibition of α-amylase and α-glycosidase enzymes. In addition, it was proposed to encapsulate the extract by complex coacervation using polymeric pairs formed between gelatin and five different polysaccharides (gum arabic, pectin, cashew gum, carboxymethylcellulose, and κ-carrageenan), as well as characterize the encapsulated extract particles. Drying of the extract caused losses in the initial content of the phenolic compounds. The dry extracts, especially the atomized sample, presented high antioxidant capacity and high potential to inhibit the digestive enzymes, but they lost the antimicrobial capacity in relation to the liquid extract. The microparticles of the encapsulated extract had high efficiency and yield for encapsulation, varied sizes, and irregular shapes. Infrared spectra demonstrated the interaction between protein and polysaccharides in the formation of encapsulated extract particles. The results indicate that the complex coacervation and the materials used have the potential to encapsulate the cinnamon extract, which can contribute to protect its bioactive compounds, their beneficial properties, and mask their undesirable sensorial characteristics.

1. Introduction

The aqueous or alcohol extracts obtained from several varieties of cinnamon (Cinnamomum spp.) are known to have several beneficial effects on health such as anti-diabetes, antioxidant, and antimicrobial properties, among others (Anderson et al., 2004; Beecher, 2004; Shan, Cai, Brooks, & Corke, 2007). Many of these benefits are attributed to the phenolic compounds present in this plant, such as proanthocyanidins, which are a class of flavonoids formed by oligomers and flavan-3-ol polymers (Ho, Rafi, & Ghai, 2010; Jiao et al., 2013).

Proanthocyanidins, as well as other phenolic compounds, may undergo degradation reactions especially when in aqueous media (Rohr, Meier, & Sticher, 2000; Munin and Edwards-Lévy, 2011). Furthermore, these compounds are responsible for presenting bitter taste and causing a strong sensation of astringency by the precipitation of salivary proteins in the mouth (Bennick, 2002; Hofmann et al., 2006). Thus, the consumption of large quantities of cinnamon extract that would be necessary to enjoy its benefits, is

* Corresponding author.
E-mail address: volnei15@gmail.com (V.B. de Souza).

https://doi.org/10.1016/j.foodhyd.2017.09.040
0268-005X/

restricted by these technological problems. Obtaining a dry extract would be interesting, as it would help to stabilize the product, reducing the water activity, in addition to concentrating the compounds of interest. However, the dry extract, due to the high concentration of proanthocyanidins, would cause strong bitterness and sensation of astringency which would imply a problem for the application and consumption of the cinnamon extract.

Microencapsulation is a technology that has been used to protect sensitive compounds from adverse environmental conditions. In addition, encapsulation may promote controlled release and mask unwanted sensory aspects of some compounds (Arshady, 1993; Fávaro-Trindade, Pinho, and Rocha, 2007; Oehme, Valotis, Krammer, Zimmermann, & Schreier, 2011). Complex coacervation is used as an encapsulation technique based on the interaction in aqueous media between two oppositely charged polyelectrolytes, where a complex between the encapsulating materials (usually proteins and polysaccharides) will form around droplets or particles of the active material (Singh & Burgess, 1989; De Kruif, Weinbreck & De Vries, 2004). It is widely used for the encapsulation of aromas and other hydrophobic compounds, however, it has the potential to encapsulate other types of compounds, such as water-soluble vitamins, sweeteners, and phenolic compounds (Alvim & Grosso, 2010; Nori et al., 2011; Comunian et al., 2013; Rocha-Selmi, Theodoro, Thomazini, Bolini, & Favaro-Trindade, 2013a, b). The encapsulation of phenolic compounds by complex coacervation is described very little in the literature (Nori et al., 2011; Strauss & Gibson, 2004). In other studies of the research group, cinnamon extract rich in phenolic compounds was encapsulated by the spray chilling technique (Tulini et al., 2016; 2017). However, to the best of our knowledge, there is no work on the encapsulation of proanthocyanidin-rich cinnamon extract by complex coacervation.

There are several polymeric materials used for encapsulation in the process of complex coacervation, and one of the most common polymeric pairs is composed of proteins and polysaccharides (De Kruif, Weinbreck & De Vries, 2004; Schmitt & Turgeon, 2011). The most widely used system for microencapsulation of different compounds by complex coacervation is formed by gelatin and gum arabic. However, other materials have been studied and present potential as encapsulants (Gomez-Estaca, Comunian, Montero, Ferro-Furtado, & Favaro-Trindade, 2016; Timilsena, Adhikarib, Barrow, & Adhikari, 2016).

The objective of the present study was to evaluate the bioactive potential of proanthocyanidin-rich extract obtained from cinnamon (Cinnamomum zeylanicum) in its liquid and dehydrated form by lyophilization and spray drying. The use of the complex coacervation technique using gelatin and five different polysaccharides for the encapsulation of the spray-dryed proanthocyanidin-rich extract, as well as the characterization of the microparticles obtained, was also proposed.

2. Materials and methods

2.1. Materials

Ceylon cinnamon (*C. zeylanicum*) in bark was obtained from WG Products (Waskaduwa, Sri Lanka). Type A swine gelatine (Gelnex, Itá, Santa Catarina, Brazil), gum Arabic (Nexira Brazil, São Paulo, Brazil), citrus pectin with high level of methoxylation (Vetec-Sigma Aldrich, St. Louis, (Brazil)), carboxymethylcellulose (Arcólor, São Lourenço, São Paulo, Brazil), k-carrageenan (kindly donated by Kerry, Três Corações, Minas Gerais, Brazil), and cashew gum (kindly donated by Embrapa Agroindústria Tropical, Fortaleza, Ceará, Brazil) were used as encapsulant materials.

2.2. Obtaining of proanthocyanidin-rich cinnamon extract

The proanthocyanidin-rich cinnamon extract (PRCE) was obtained according to Souza et al. (2017) (unpublished results). Ceylon cinnamon in crushed bark and aqueous ethanol (50% by mass) solvent were used in the ratio of 1:7.5 between solid:solvent. Extraction was performed at 60 °C for 30 min. The liquid extract was obtained after filtration in a cotton cloth to remove the larger particles of the cinnamon bark and centrifugation for 5 min at 25 °C and 6603 g in a Model 5430 centrifuge (Eppendorf, Hamburg, Germany).

2.3. Drying of PRCE by spray drying and lyophilization

The PRCE was dehydrated by spray drying without any excipient in a spray dryer model MSD 1.0 (Labmaq of Brasil Ltda, Ribeirão Preto, Brazil), using inlet air temperature of 150 °C with air drying velocity of 2.5 m/s, feeding flow rate of 16 mL/min guaranteed through a peristaltic pump model Labmaq OS-1 (Labmaq do Brasil Ltda, Ribeirão Preto, Brazil). The spray nozzle double fluid of 1.2 mm and compressed air flow of 40 L/min were used. The spray-dried PRCE (SD-PRCE) was stored in a polyethylene bottle covered with foil and stored in a freezer at −20 °C until use.

In order to obtain the freeze-dried PRCE (FD-PRCE), the ethanol present was removed by means of the TE-211 rotary-evaporator concentration (Tecnal, Piracicaba, Brazil) at 40 °C until the initial volume was reduced by half. The concentrated extract (C-PRCE) was frozen at −20 °C and dehydrated in a freeze drier model LC 1500 (Terroni, São Carlos, Brazil) for 24 h, without any excipient. The sample was conditioned and stored under the same conditions described for SD-PRCE.

2.4. Phenolic compounds and functional properties of PRCE's

For these tests, the ethanolic (PRCE), concentrated (C-PRCE), atomized (SD-PRCE), and lyophilized (FD-PRCE) extracts were evaluated.

2.4.1. Total proanthocyanidins

The total proanthocyanidins content was determined according to Payne et al. (2010) using the 4-dimethylaminocinnamaldehyde (DMAC) method with some modifications. 0.5 ml of the diluted extracts were added in threaded tubes. At the time of analysis, 2.5 ml of the DMAC reagent (0.001 g/ml in absolute ethanol acidified with 10% concentrated HCl) were added. The absorbance was monitored on a DR 2800 HACH (Loveland, USA) spectrophotometer at 640 nm for 5 min at 10 s intervals in order to obtain the maximum absorbance value. Procyanidin B2 (Sigma-Aldrich, St. Louis, United States) was used as the reference substance and the results were expressed as mg equivalents of procyanidine B2/g extract on a dry basis.

2.4.2. Total phenolics

The total phenolic content was quantified by the Folin-Ciocalteu method according to Singleton, Orthofer, and Lamuela-Raventos (1999) with minor modifications. 250 μL of the diluted extracts were added to a glass tube threads. Subsequently, 250 μL of the Folin-Ciocalteu reagent (Dinâmica Química Contemporânea Ltda., Diadema, Brazil) and 2 mL of distilled water were added. After 3 min at room temperature, 250 μL of a saturated sodium carbonate solution (30 g/100 mL) were added, and the mixture was maintained at 37 °C in a water bath for 30 min. The absorbance was measured at 750 nm using a spectrophotometer (model DR 2800 HACH, Loveland, USA). Gallic acid was used as the reference standard, and the results were expressed as mg equivalent of gallic acid/g of extract on a dry basis.

2.4.3. Sequestration capacity for DPPH radicals

The sequestration capacity of DPPH• (2,2-diphenyl-1-picrylhydrazyl radical) was verified according to Brand-Williams, Cuvelier, and Berset (1995) with modification in total reaction volume where there was adaptation to a microplate reader. Initially, 50 μL aliquots of the diluted extracts were mixed in a 96-well microplate with 250 μL of a DPPH• (0.5 mmol/L) methanolic solution. The mixture was stirred and after 25 min at 25 °C, the absorbance was measured at 517 nm using a Microplate Spectrophotometer (Benchmark Plus, Biorad, Hercules, CA, USA). A standard stock solution (Trolox 0.25 mg/mL ~ 1000 μmol/L) at concentrations of 20, 30, 40, 50, 60, 70, and 80 μmol/L. The DPPH• radical sequestering capacity was expressed as μmol Trolox equivalents/g extract on a dry basis.

2.4.4. Iron reduction capacity (FRAP)

This assay was performed according to Benzie and Strain (1996). 20 μl of the diluted extracts were used. The absorbance was measured at 593 nm using a Microplate Spectrophotometer (Benchmark Plus, Biorad, Hercules, CA, USA). A standard stock solution of Trolox (0.25 mg/mL ~ 1000 μmol/L) at concentrations of 50, 100, 200, 300, and 400 μmol/L was used as a reference, and the results were expressed as μmol equivalents of Trolox/mL of the fraction.

2.4.5. Inhibitory activity of α-amilase

Inhibition of the α-amylase enzyme by the PRCE samples was evaluated according to the modified chromogenic method of the Whorthington Enzyme Manual (Worthington, 1993) in order to indicate the type 2 anti-diabetes capacity of the extracts. Approximately 0.1 g of the extracts were diluted in a 10 mL volumetric flask using 50% ethanol (v/v). Then 0.5 mL of the first dilution was dissolved in 25 mL volumetric flask using the same solvent. Aliquots of diluted extracts were used in the volumes of 5–100 μL, and the volume was filled to 250 μL with 0.1 M potassium phosphate buffer at pH 6.9 containing 0.006 M NaCl. 250 μL of the solution (1 U/mL) of the enzyme porcine pancreatic α-amylase (EC 3.2.1.1, type VI-A, Sigma Chemical Co., Saint Louis, USA) was added to each tube and incubated at 25 °C for 10 min. Then 250 μL of the potato starch solution (1%) was added. Again, the tubes were incubated at 25 °C for 10 min. The reaction was stopped by the addition of 250 μL of a solution of 3,5-dinitrosalicyclic acid (DNS). The tubes were brought to the boiling bath at 100 °C for 10 min and then cooled to room temperature. 4 mL of distilled water were added to each tube, and then the absorbance was read at 540 nm in U-1100 spectrophotometer (Hitachi, Japan). The readings were compared to the control containing phosphate buffer in place of the sample (control Abs) and also with a blank containing phosphate buffer in place of the enzyme (blank sample). The enzyme inhibition percentage for each sample concentration was calculated according to Equation (1), and the results were expressed as sample concentration (μg of extract b.s./mL of reaction) required to inhibit 50% enzyme activity (IC 50).

$$\%inhibition = \left(\frac{\left(Abs_{control} - \left(Abs_{sample} - Abs_{blank\ sample} \right) \right)}{Abs_{control}} \right) \times 100 \quad (1)$$

2.4.6. Inhibitory activity α-glicosidase

Inhibition of α-glycosidase enzyme by PRCE samples was measured according to the method proposed by Mccue, Kwon, and Shetty (2005) also in order to indicate the type 2 anti-diabetes capacity of the extracts. Approximately 0.1 g of the extracts were diluted in a 10 mL volumetric flask using 50% ethanol (v/v). Then 0.5 mL of the first dilution was dissolved in 25 mL volumetric flask using the same solvent. Aliquots of the previously diluted extracts were added in a 96 well microplate in volumes of 1–10 μL, and the volume was completed to a total 50 μL with 0.1 M potassium phosphate buffer at pH 6.9. 100 μL of the *Saccharomyces cerevisae* α-glycosidase enzyme solution (1 U/mL) (EC 3.2.1.20, Sigma Chemical Co., Saint Louis, USA) was added to each well containing the samples in phosphate buffer. The plate was incubated at 25 °C for 10 min, and then, 50 μl of the substrate solution *p*-nitrophenyl-α-glucopyranoside (*p*-NPG, Sigma Chemical Co., Saint Louis, USA) were added to 1.5 mg/mL in phosphate buffer. The 1st absorbance reading (0 min) was performed at 405 nm, and the plate was again incubated at 25 °C for 5 min. Then, the 2nd absorbance reading (5 min) was performed at 405 nm. Reaction and reading were performed on the Synergy H1 microplate reader (BioTek Instruments Inc., Winooski, VT, United States). The readings were compared to the control containing 50 μl of phosphate buffer in place of the extracts. The enzyme inhibition percentage for each sample concentration was calculated according to Equation (2), and the results were expressed as the concentration of sample (μg extract b.s./mL reaction) required to inhibit 50% enzyme activity (IC 50).

$$\%inhibition = \left(\frac{(Abs_{control\ 5min} - Abs_{control\ 0min}) - (Abs_{extract\ 5min} - Abs_{extract\ 5min})}{(Abs_{control\ 5min} - Abs_{control\ 0min})} \right) \quad (2)$$

2.4.7. Antimicrobial activity

Initially, the agar diffusion method (CLSI, 2009a) was screened, and minimal inhibitory and bactericidal concentrations were determined (CLSI, 2009b). The liquid extracts were evaluated without dilution, and the dried extracts were tested at the concentration of 0.5 g/mL against the Gram-positive bacteria *Staphylococcus aureus* subsp. *aureus* CCT 5591 and *Listeria monocytogenes* CCT 7408 and the Gram-negative bacteria *Salmonella enteritidis* CCT 4475 and *Escherichia coli* CCT 0548. All cultures were kindly donated by the Tropical Cultures Collection of the André Tosello Foundation (Campinas, Brazil).

2.5. Encapsulation of SD-PRCE by complex coacervation

Particles containing SD-PRCE were obtained by the complex coacervation technique, as described by Nori et al. (2011) with some modifications. Initially, aqueous solutions of encapsulating materials were prepared in the following concentrations: gelatin (GEL - 5 g/100 mL), gum Arabic (GA - 5 g/100 mL), cashew gum (CG

- 5 g/100 mL), pectin (PEC - 2.5 g/100 mL), κ-carrageenan (CARR - 2.5 g/100 mL), and carboxymethylcellulose (CMC-1 g/100 mL). The parameters used for encapsulation (ratio of polymers, pH, and core concentration) are shown in Table 1. The pH and core concentration values were determined in preliminary tests (data not shown). The proportions between the polymers for the systems containing pectin and carrageenan were also defined in preliminary tests. For the gum Arabic system, the proportion based on previous work by the group (Comunian et al., 2013), as well as for cashew gum (Gomez-Estaca et al., 2016), was used. For the CMC system, the ratio was defined by Lv, Zhang, Abbas, and Karangwa (2012).

To promote encapsulation, a mass of SD-PRCE, as described in item 2.3, was dispersed in distilled water (twice the volume of total polymer solutions) using a T25 Digital Ultra Turrax (IKA, Staufen, Germany) at 6000 rpm and 40 °C for 2 min. The gelatin dispersion was added, and the spin was increased to 12000 rpm for 2 more min. Then, the polysaccharide solution was added in the respective ratio cited above, relative to the gelatin, by stirring for another 2 min at the same rate. The system was then kept under magnetic stirring at 40 °C where the pH was adjusted with a 1M HCl solution. The system was then cooled to 10 °C in an ice bath. The particles were allowed to settle overnight at 4 °C, and then, the supernatant was removed. The decanted material was housed in Petri dishes, frozen at −20 °C, and dried in freeze-dryer model LC 1500 (Terroni, São Carlos, Brazil) for 24 h. The obtained samples were conditioned in bags of high density polyethylene and stored at −20 °C for the characterization analyzes.

2.6. Efficiency and yield of encapsulation

To determine the efficiency and encapsulation yield, 0.1 g of the dried particles were weighed into tubes, and 2.5 μL of a 0.1 M NaOH solution were added. The tubes were vigorously vortexed with vortex model AV-2 (Gehaka, São Paulo, Brazil) until the dissolution of the complex was complete to obtain a homogeneous solution. The solution was then transferred to a 25 mL volumetric flask, and the volume was completed with distilled water. After dilution, this solution was used for the quantification of total phenolic compounds by the reduction of Folin-Ciocalteu reagent method of as described in item 2.4.2. To determine the phenolic compounds on the surface of the particles, 0.1 g of the samples were weighed into tubes, and 5 mL of distilled water were added. The tubes were vortexed rapidly and then centrifuged for 5 min at 10 °C and 6603 g in a model 5430 centrifuge (Eppendorf, Hamburg, Germany). The supernatant was collected in a 25 mL volumetric flask, and the volume filled with distilled water.

The encapsulation efficiency (EE) was calculated according to Equation (3), and the encapsulation yield (EY) was obtained according to Equation (4).

$$EE(\%) = \left(\frac{Phenolics_{exp} - Phenolics_{sur}}{Phenolics_{theo}}\right) \times 100 \quad (3)$$

Table 1
Parameters determined for the production of the microparticles containing SD-PRCE encapsulated by complex coacervation using different wall materials.

Polimeric pair	Gelatin: polysaccharide ratio	pH	Active (SD-PRCE) concentration (% w/w)
Gelatin:Gum Arabic	1:1	3.8	25%
Gelatin:Pectin	2:1	3.5	25%
Gelatin:Cashew gum	1:2.5	4.1	25%
Gelatin: Carboxymethylcellulose	7:1	4.4	30%
Gelatin:κ-carrageenan	4:1	4.2	35%

$$EY(\%) = \left(\frac{Phenolics_{exp}}{Phenolics_{theo}}\right) \times 100 \quad (4)$$

Where:

Phenolics $_{exp}$ - total phenolic concentration determined in microparticles experimentally.
Phenolics $_{sur}$ - phenolic concentration determined on the surface of the microparticles after washing.
Phenolics $_{theo}$ - theoretical amount of phenol added at the beginning.

2.7. Morphology

The particle morphology was assessed by optical microscopy analysis using an optical microscope (BEL Photonics, Milan, Italy), and images were collected with the camera attached to the equipment and aid from BELView software (BEL Photonics, Milan, Italy). Images of the wet, newly produced particules were collected with an increase of 40–100 times magnification.

The dehydrated particles of both free extract and encapsulated samples were evaluated by Scanning Electron Microscopy (SEM) on a Tabletop Microscope ™ 3000 electronic microscope (Hitachi, Tokyo, Japan). The samples were fixed in metallic supports (*stubs*) with conventional double-sided adhesive tape, and this system was taken under a microscope. The acceleration voltage of 15 KV electrons was used, and the images were acquired with a 1000-fold increase using the TM 3000 software.

2.8. Particle size

The average particle diameter of the core material (SD-PRCE) was obtained by laser diffraction analysis in SALD/201V model equipment (Shimadzu, Kyoto, Japan) with a measurement range of 0.5–500 micrometers. For this analysis, a small amount of this sample was dispersed in liquid Vaseline, obtaining a suspension, and submitted to 4 readings. The diameter D [4,3] was obtained and recognized as the diameter of “de Brouckere”. For the encapsulated extract microparticles, obtaining the mean diameter was possible through image analysis using ImageJ Software, following the methodology proposed by Marcomini and Souza (2011). For each sample, 10 images obtained by optical microscopy were used, and the mean diameter of Feret was obtained for these particles. The span value $((d_{(0,9)} - d_{(0,1)})/d_{(0,5)})$, which gives an idea of the size distribution width, and the polydispersity of the system was calculated considering the mean diameter in reference to 10, 50, and 90% of the total particles (Ilic et al., 2009).

2.9. FTIR analysis

Infrared spectra were acquired for the SD-PRCE samples, being for the polymers used as wall material, as well as for the microparticles containing the encapsulated extract. This analysis was performed in FT-IR Spectrometer equipment (Perkin Elmer, Massachusetts, USA) with the aid of Spectrum One software version 5.3.1. The spectra were obtained in the region of 4000 to 600 cm^{-1}, and each spectrum was the result of 16 readings.

2.10. Statistical analysis

Analysis of Variance (ANOVA) and Tukey averages comparison test were performed, considering that there was a significant difference between the samples when $p < 0.05$. These analyzes were

performed to verify the statistical difference between the samples in the analyses of antioxidant and anti-diabetes capacity of the extracts, as well as in the characterization of the microparticles. All statistical procedures were performed using the SAS statistical package (version 9.2., SAS Institute Inc., Cary, NC, United States).

3. Results and discussion

3.1. Antioxidant, anti-diabetes and antimicrobial properties of liquid and dried PRCE

Cinnamon extracts from different species and obtained with different solvents are known to have several beneficial effects to health. For example, the methanol extract of *Cinnamomum burmanii* presented an antimicrobial effect against some pathogenic bacteria (Shan et al., 2007). The same variety extracted with acidified water showed anti-type 2 diabetes effect in vitro (Anderson et al., 2004). Infusions obtained from *C. zeylanicum* with water at 80 °C and dried by spray drying with maltodextrin presented antioxidant capacity according to Santiago-Adame et al. (2015). Table 2 shows the results of total proanthocyanidins and phenolics, antioxidant capacity, and inhibitory activities of α-amylase and α-glycosidase for PRCE obtained from *C. zeylanicum* using aqueous ethanol as the solvent and dried by spray drying and freeze-drying.

The PRCE drying processes promoted losses in the total proanthocyanidin content of the extract. The values were statistically different and losses of 6, 17, and 23% of the initial contents of proanthocyanidins were seen in the concentrated, atomized, and lyophilized extracts, respectively. The same behavior was observed for the total phenolic content. Thermal processes, such as concentration and spray drying, are responsible for the degradation of sensitive compounds, such as phenolics. Although the temperature used during the extract concentration was not high (40 °C), the exposure time at that temperature was sufficient to promote loss of these compounds, even if small. In addition, among the drying methods, lyophilization proved to be worse than spray drying to obtain powdered PRCE. The total losses in lyophilization were due, in part, to the previous process of extract concentration and also to a long drying time (24 h), even at low temperatures and low oxygen content. Other factors, such as the incidence of light, could have contributed to the degradation of phenolic compounds in this process.

Regarding the antioxidant capacity, there was no significant difference between the drying methods, both of which promoted similar losses in both the sequestering capacity of DPPH radicals and in the reducing capacity of the iron when comparing to the samples of the liquid extracts. These losses are also related to the drying conditions, such as high temperature, process time, exposure to light and oxygen, among others that may influence the degradation of the compounds responsible for these activities. Losses in phenolic content and antioxidant capacity due to drying processes were also observed by Fujita, Borges, Correia, Franco, and Genovese (2013) when evaluating camu-camu extracts dried by lyophilization and spout bed when compared to fresh fruit pulp. Proanthocyanidins are the main phenolic compounds present in the aqueous extract of cinnamon (Nonaka, Morimoto, & Nishioka, 1983). These molecules are also mainly responsible for the antioxidant capacity of the extract (Gruenwald, Freder, & Armbruester, 2010).

The anti-diabetes type 2 effect is a property presented by the aqueous and alcoholic extracts of different varieties of cinnamon (Anderson et al., 2004; Jiao et al., 2013; Khan, Safdar, Ali-Khan, Khattak, & Anderson, 2003). This activity is focused on the inhibition of the α-amylase and α-glycosidase digestive enzymes responsible for the digestion of soluble carbohydrates. For the inhibition of these enzymes, in contrast to the antioxidant capacity, the drying processes of the extract, especially spray drying, potentiated its action in the inhibition of digestive enzymes. At first, this seems to be a conflicting result because, as has been observed, drying processes have caused loss in the total content of proanthocyanidins, and these molecules are known to be responsible for the ability of cinnamon to inhibit α-amylase and α-glycosidase (Anderson et al., 2004; Anhê, 2013). However, this can be explained by the fact that the drying processes used, especially spray drying, promote high loss of the volatile compounds present in the PRCE, such as cinnamaldehyde (data not shown). Thus, with the considerable loss of mass of these volatile compounds, the proanthocyanidins represent a larger fraction of the total compounds present in the extract.

The ability to inhibit the multiplication of pathogenic microorganisms has already been observed in lyophilization dehydrated cinnamon extract (*Cinnamomum burmannii*) (Shan et al., 2007). In the present work, the antimicrobial activity against the pathogenic bacteria *Staphylococcus aureus, Listeria monocytogenes, Salmonella enteritidis* and *Escherichia coli* was evaluated, and it was observed that the PRCE and C-PRCE were the groups that showed the greatest capacity of inhibition of the microorganisms tested, presenting effect against all of them. SD-PRCE inhibited the growth of L. monocytogenes whereas FD-PRCE showed no inhibitory effect against any of the microorganisms evaluated. The considerable decrease of this capacity in the dry extracts may be linked to the loss of cinnamaldehyde during drying. This volatile compound is commonly identified as one of the main constituents of the cinnamon extract and, according to Shan et al. (2007), is the compound that most contributes to the antimicrobial capacity of *C. burmannii* extract. In addition, the antimicrobial potential of cinnamon appears to be mainly related to its essential oil, in which cinnamaldehyde is present in large quantities (Gruenwald et al., 2010).

Based on the results obtained in the assessment of the bioactive potential of dry and liquid cinnamon extracts, SD-PRCE was chosen to be encapsulated by complex coacervation. This extract had high inhibitory activity of α-amylase and α-glycosidase enzymes and also had a high concentration of proanthocyanidins (approximately 35% on dry basis). In addition, it requires less time and steps to obtain.

Table 2
– Total content of proanthocyanidins (mg eq procyanidin B2/g of extract dry basis), Folin-Ciocalteu reduction power (mg eq. galic acid/g of extract dry basis), antioxidant capacity by DPPH and FRAP (μmol eq. Trolox/g of extract dry basis) and inhibitory activities (IC50) of α-amylase and α-glucosidase (μg/mL of reaction medium) for the Ceylon cinnamon extracts.

Sample	Total proanthocyanidins *	Folin-Ciocalteu reduction power *	DPPH *	FRAP *	Inibition of α-amylase *	Inibition of α-glucosidase *
PRCE	419 ± 4 [a]	740 ± 8 [a]	2304 ± 62 [a]	1277 ± 87 [a]	4.1 ± 0.2 [a]	6.3 ± 0.1 [a]
C-PRCE	395 ± 4 [b]	698 ± 8 [b]	2249 ± 62 [a]	1240 ± 87 [a]	3.7 ± 0.2 [a]	6.2 ± 0.1 [a]
SD-PRCE	346 ± 8 [c]	610 ± 10 [c]	2072 ± 32 [b]	924 ± 46 [b]	2.6 ± 0.2 [b]	0.8 ± 0.0 [b]
FD-PRCE	321 ± 5 [d]	567 ± 5 [d]	2055 ± 42 [b]	860 ± 43 [b]	3.5 ± 0.3 [a]	0.7 ± 0.0 [b]

*Mean ± standard deviation. Averages in the same column and followed by different letters differ from each other ($p < 0.05$). N = 3 replicates.

3.2. Characterization of microparticles containing SD-PRCE

The microparticles were obtained by complex coacervation according to the parameters presented in Table 1. These parameters were defined in preliminary tests that took into account the morphology of the particles as well as the total proanthocyanidins content that were quantified in the coacervation water (data not shown).

3.2.1. Efficiency and yield of encapsulation

Efficiency and yield are important parameters in the development of an encapsulation process, regardless of the method and the substance to be encapsulated. Typically, hydrophilic materials are not encapsulated directly by the complex coacervation technique, being it necessary to obtain an emulsion of this material in oil. This emulsion is then used as a core to obtain the particles (Comunian et al., 2013; Rocha-Selmi, Bozza, Thomazini, Bolini, & Fávaro-Trindade, 2013b, 2013a; Santos, Bozza, Thomazini, & Favaro-Trindade, 2015). Such a step is necessary because the encapsulation efficiency of hydrophilic substances tends to be much lower than that of lipophilic substances, since the former tend to migrate to the aqueous phase during the coacervation process. In the present study, the SD-PRCE obtained from the *C. zeylanicum* variety was encapsulated using the complex coacervation technique without the use of primary emulsion and using different polymer pairs, as already described. The phenolic and total proanthocyanidins contents, as well as the encapsulation efficiency and yield for each type of particle, were evaluated, and the results are presented in Table 3.

The concentrations of the phenolic compounds in the particles were variable, since, different concentrations of cores were used for each polymer pair, in addition to different proportions among the polymers.

High values of efficiency and yield for encapsulation were obtained for hydrophilic compounds and direct encapsulation. This result may be related to the ability of phenolic compounds, especially proanthocyanidins, to bind proteins, such as gelatin. The interaction of the compounds in the extract with the gelatin, used as wall material, may have contributed to increase the amount of the phenolic compounds that were encapsulated.

Nori et al. (2011) obtained particles of propolis extract, which is also rich in phenolic compounds, by complex coacervation using soy protein isolate and pectin as encapsulating materials. These authors started from the liquid extract but used a methodology similar to the one presented here and obtained encapsulation efficiency between 66 and 72%, values, which are similar to those found in the present study. Santos et al. (2015) obtained encapsulation efficiencies ranging from 31 to 63% for xylitol (highly hydrophilic) encapsulated using double emulsion followed by complex coacervation with gelatin/gum Arabic as encapsulants, which are values smaller than those obtained in the present study. García-Saldaña et al. (2016), using gelatin/pectin to encapsulate sulforaphane from broccoli seeds by complex coacervation, obtained encapsulation yield of 81% and efficiency of 18%, which are lower values than those found for SD-PRCE encapsulated with the same materials.

3.2.2. Morphology of particles

The SD-PRCE particles presented a spherical shape, being characteristic of dry materials in the spray dryer, as can be observed in the image obtained by scanning electron microscopy (Fig. 1).

In the present work, all the particles obtained with the different encapsulating materials presented an irregular shape, as can be observed both in the images obtained by optical microscopy and those obtained by SEM (Fig. 1). Normally, particles obtained by complex coacervation are spherical in shape (Comunian et al., 2013; Silva, Favaro-Trindade, Rocha, & Thomazini, 2012). The irregular shape observed in the present study may be related to the interaction between PRCE and wall material, mainly gelatin, which are caused by the presence of a large amount of proanthocyanidins in the extract. It was observed that with lower extract concentration (therefore less proanthocyanidins), especially in the gelatin/gum Arabic and gelatin/pectin systems, the particles presented a more regular shape with more defined walls (Fig. 1; b and c).

Lv et al. (2012) obtained spherical particles containing jasmine essential oil using gelatin/CMC as encapsulants with the same protein to polysaccharide and pH ratio values that were used in the present work, which reinforces the idea that the type of active material may influence the morphology of the particles obtained.

This irregular shape for particles containing extracts rich in phenolics was also observed by Nori et al. (2011) when studying soy protein isolate/pectin particles encapsulating propolis extract. Gomez-Estaca et al. (2016) observed irregular shape in particles obtained with gelatin/cashew gum encapsulating shrimp astaxanthin. These results are similar to those obtained by Comunian et al. (2016) when encapsulating Echium oil also with gelatin/cashew gum. This means that the shape of the particles may also be related to the type of wall material used.

3.2.3. Particle size

Particle size is an important parameter, especially from the point of view of the application of food ingredients, since very large particles can be undesirable when causing an unpleasant mouthfeel. In other cases, they may be desired, like when the goal is to obtain products where the particles are visible. In addition, large particles can allow a prolonged release of the active compounds (Trinh, Shaari, Basit, & Azeem, 2014). And this would ensure its beneficial actions for an extended time. The mean particle size for samples of free SD-PRCE and also for encapsulated SD-PRCE particles was measured and the results are presented in Table 4.

It is possible to observe that the SD-PRCE presented the smallest mean particle size (D 4.3), while the particles of the encapsulated extract presented different sizes (D Feret). In the encapsulation process, there is the addition of polymeric coating on the extract, and in addition, the extract particles are not encapsulated

Table 3
Phenolic compounds, total proanthocyanidins, efficiency and encapsulation yield for the particles containing SD-PRCE encapsulated by complex coacervation using different wall materials.

Sample	Total phenolics (mg eq galic acid/g)*	Total proanthocyanidins(mg eq procyanidin B2/g)*	Encapsulation efficiency (%)*	Encapsulation yield (%)*
GEL/GA	49 ± 1^{c}	21.2 ± 0.7^{c}	73 ± 3^{b}	80 ± 3^{b}
GEL/PEC	75 ± 6^{b}	38 ± 6^{b}	85 ± 2^{a}	92 ± 2^{a}
GEL/CG	124 ± 8^{a}	69 ± 7^{a}	86 ± 6^{a}	91 ± 6^{a}
GEL/CMC	55 ± 2^{c}	24.5 ± 0.6^{c}	$67 \pm 1^{b,c}$	73 ± 2^{c}
GEL/CARR	42 ± 13^{c}	15 ± 6^{c}	65 ± 4^{c}	70 ± 4^{c}

*Mean ± standard deviation. Averages in the same column and followed by different letters differ from each other ($p < 0.05$). N = 3 replicates.
GEL - Gelatin; GA – Gum Arabic; PEC – Pectin; CG – Cashew Gum; CMC – carboxymethylcellulose; CARR - κ-carrageenan.

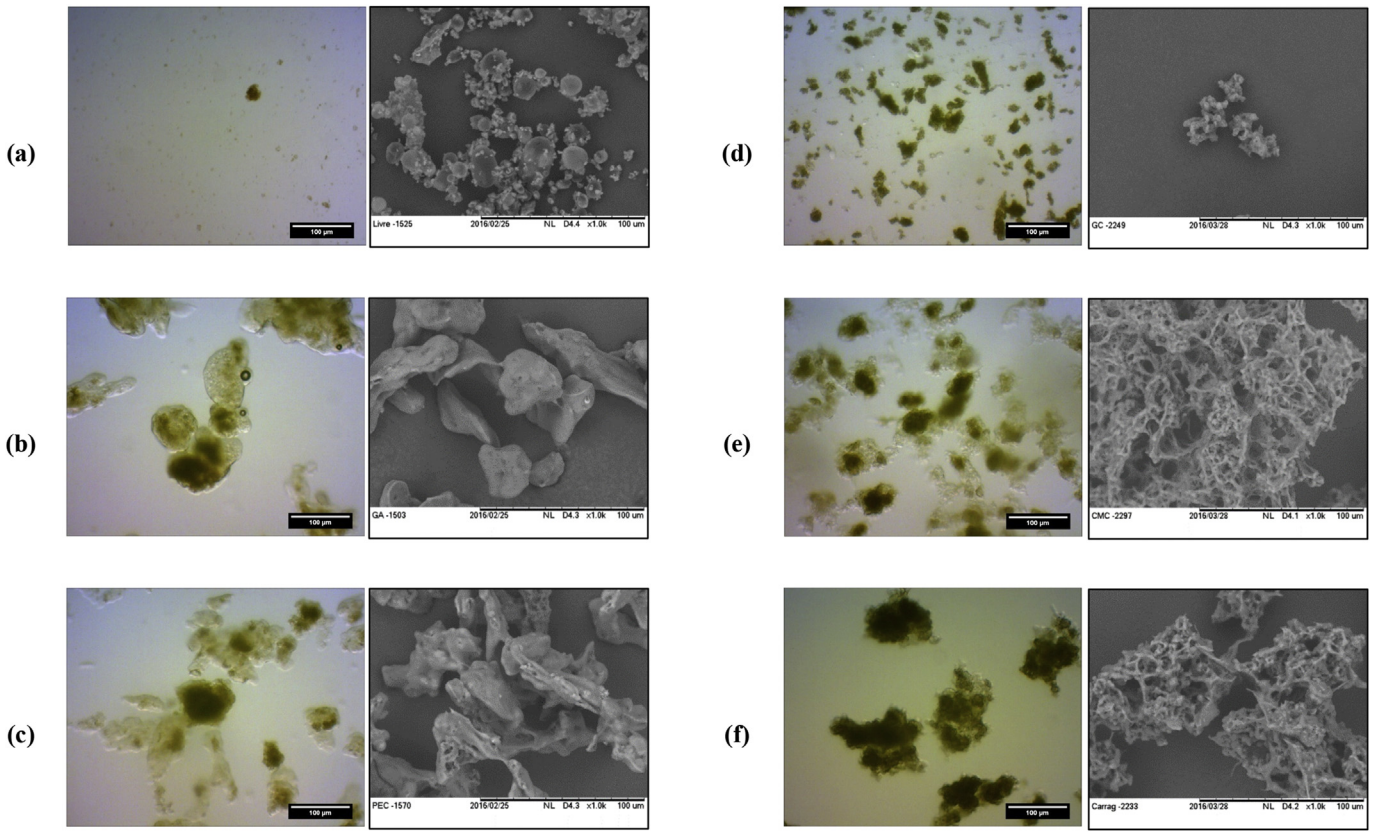

Fig. 1. Optical microscopy and scanning electron microscopy (SEM) of the spray-dried proanthocyanidin-rich cinnamon extract (SD-PRCE) and microparticles containing the SD-PRCE encapsulated by complex coacervation. (a) free extract; (b) particle produced with GEL/AG; (c) particle produced with GEL/PEC; (d) particle produced with GEL/CG; (e) particle produced with GEL/CMC; (f) particle produced with GEL/CARR. Optical microscopy image (increase of 100 times) and SEM (increase of 1000 X).

Table 4
Mean particle size and span value for spray-dried cinnamon extract and for the extract particles encapsulated by complex coacervation with different wall materials.

Sample	Mean diameter (μm)	Span value
Free SD-PRCE	7.6 ± 0.1 ^{d}	0.3
GEL/GA	121 ± 24 $^{a, b}$	1.0
GEL/PEC	116 ± 10 ^{b}	1.2
GEL/CG	26 ± 3 ^{c}	2.0
GEL/CMC	29 ± 7 ^{c}	3.1
GEL/CARR	149 ± 21 ^{a}	1.9

*Mean ± standard deviation. Averages in the same column and followed by different letters differ from each other ($p < 0.05$). N = 3 replicates.
GEL - Gelatin; GA – Gum Arabic; PEC – Pectin; CG – Cashew Gum; CMC – carboxymethylcellulose; CARR - κ-carrageenan.

individually but together, resulting in multinucleated structures.

Rocha-Selmi et al. (2013b) obtained aspartame microcapsules by complex coacervation with gelatin/gum arabic, and the particles had sizes between 84 and 102 μm. Dai et al. (2010) encapsulated complex coacervation electrophoresis fluid using gelatin/carboxymethylcellulose/dioctyl sulfosuccinate sodium. They obtained particles with a mean diameter of 36 μm. In these two studies, were used similar wall materials to those used in the present study. However, the authors measured the diameter by laser diffraction in both cases and did not present the reference diameter. It is not common to use image analysis for particles obtained by complex coacervation. Probably because most of the times the capsules of lipophilic materials commonly obtained by this technique have spherical shape and the diameter can be measured by other methods.

The encapsulation process also caused increased polydispersity of the particles, as can be seen by the span value (Table 4). All values for the encapsulated extract were higher than for the free SD-PRCE, showing that the encapsulated SD-PRCE containing particles presented different sizes in the same sample. According to Ilic et al. (2009), higher span values mean a wider particle distribution. The free extract presented a narrower distribution with span of 0.3, and between the particles obtained by complex coacervation, the one that presented smaller polydispersity was obtained with gelatin and gum arabic.

3.3. FTIR analysis

Infrared spectra analyses can provide information about the interactions involved in the complex coacervation process between gelatin, polysaccharides, and SD-PRCE. The spectra of the separated ingredients, as well as the microparticles obtained with gelatin/CMC, are shown in Fig. 2. The other microparticles presented similar behavior (supplementary material).

The spectrum of SD-PRCE (free extract) presents a band at 3330 cm^{-1}, which is characteristic of hydroxyl groups (-OH), and, according to Ping, Pizzi, Guo, and Brosse (2012) and Santiago-Adame et al. (2015), may belong to phenolic compounds and carbohydrates. This signal was also observed in the spectra of the polysaccharides studied with small variations in the wave number. The band appearing at 2926 cm^{-1} in the free extract spectrum is due to the drawability of the -CH_2 binding (Ping et al., 2012). In the range of 1600 to 900 cm^{-1}, bands related to phenolic compounds appeared, for example proanthocyanidins. The two bands appearing at 1606 and 1519 cm^{-1} are due to the vibration of the C=C bond,

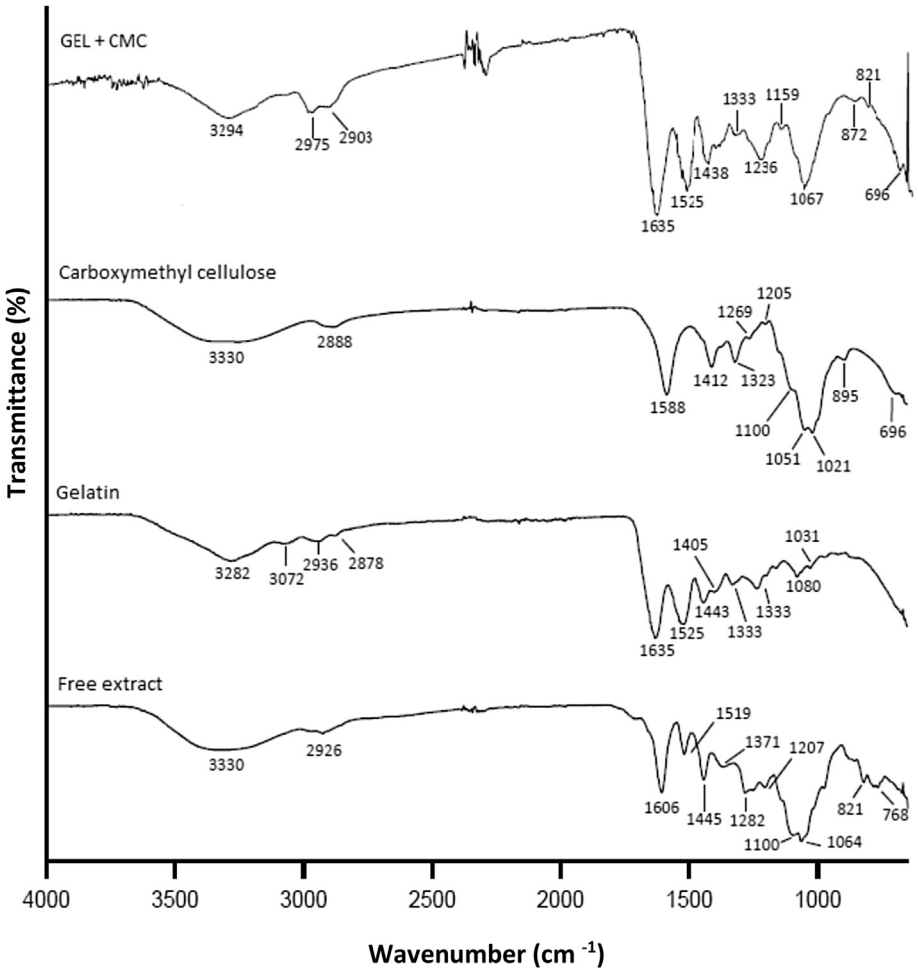

Fig. 2. Infrared spectra for the ingredients and for particles of SD-PRCE encapsulated by complex coacervation, using gelatin and carboxymethylcellulose as wall materials. For further details on the other samples, see supplemental information.

which is present in large amounts in aromatic compounds, such as phenolics (Edelmann, Diewok, Schuster, & Lendl, 2001; Souza, Thomazini, Balieiro, & Favaro Trindade, 2015; Santiago-Adame et al., 2015). According to Ping et al. (2012), the peaks that appear around 1282, 1251, and 1207 cm^{-1}, as observed in the spectrum of the free extract, are due to the presence of flavonoid tannins, which are proanthocyanidins.

By analyzing the gelatin spectrum, the presence of the following four characteristic regions is observed as described by Anvari and Chung (2016): The bands at 3282 cm^{-1} (amide A, due to the stretches of -OH and NH), at 3000-3100 cm^{-1} (amide B, due to stretches in =C-H and $-NH_3^+$), at 1600-1700 cm^{-1} (amide I, due to C=O stretching and NH bending), and at 1335-1560 cm^{-1} (amide II, due to bending of N-H and stretching of C-N). Anvari and Chung (2016) observed the band corresponding to amide I at 1635 cm^{-1}, which was exactly the same as observed in the present work (Fig. 2). These authors indicate that this value is linked to the secondary structure of the "randomly coiled" type protein, which would be a kind of favorable structure for the interaction between biopolymers in the process of complex formation.

The polysaccharides studied presented characteristic stretching bands of the typical carbohydrate -OH binding and also some bands characteristic of the stretching of the carboxylic acid salt $-COO^-$ (Fig. 2 and supplementary material). For carboxymethylcellulose (CMC), the bands that appear quite pronounced at 1588 and 1412 cm^{-1} are related, respectively, to the asymmetric and symmetrical stretches of the carboxyl group -COO-, as observed by Sitthichai, Pilapong, Thongtem, and Thongtem (2015).

For the encapsulated SD-PRCE spectrum, there was an absence of the band corresponding to the $-NH_3^+$ group (amide B of gelatin) appearing at 3072 cm^{-1} in the gelatin spectrum and also in the bands related to the stretching of the carboxyl group $-COO^-$ (1600–1415 cm^{-1}) in the polysaccharides, indicating that there was an electrostatic interaction between the amino groups ($-NH_3^+$) of the gelatin and the carboxylic groups ($-COO^-$) present in the polysaccharides studied. This behavior was also observed by Anvari and Chung (2016).

The presence of SD-PRCE in the microparticles can be seen mainly by the band around 1067 cm^{-1} from the extract, which refers to the stretching of the C-O bond present in the proanthocyanidins, as shown by Ping et al. (2012). In addition, changes in the wavelength number of the bands related to amides A, I, and II in the coacervates compared to gelatine may indicate interaction mainly of the hydrogen bonding type between phenols present in SD-PRCE. Anvari and Chung (2016) observed changes in these bands by using tannic acid as a crosslinking agent in the particles produced with fish gelatin/gum arabic. The authors attributed this result to the interaction between tannic acid and gelatin.

4. Conclusions

The spray drying and lyophilization processes of proantocyanidin-rich cinnamon extract (PRCE) caused losses in the phenolic compounds and their antimicrobial capacity compared to the liquid extract. However, there was an improvement in the antioxidant capacity and inhibition of the α-amylase and α-glycosidase digestive enzymes, especially in dry extract from spray drying. In addition, SD-PRCE presented a high concentration of phenolic compounds and proanthocyanidins on a dry basis, indicating its great potential for use as a bioactive ingredient.

In the present work, SD-PRCE was encapsulated by complex coacervation using gelatin and different polysaccharides as wall materials. The particles obtained with the different materials presented high values of efficiency and encapsulation yield, taking into account that the encapsulated active was an extract with high solubility in water and mixed directly to the polymers. The interaction between the polymers to form the wall of the capsules was confirmed by the FTIR analysis, providing further evidence that the technique chosen and the materials used were capable of successfully encapsulating SD-PRCE, which could guarantee protection of the extract compounds. This would also maintain its bioactive potential and also mask its undesired sensorial characteristics, allowing its application as a functional ingredient in food and as a nutraceutical.

Acknowledgements

We would also like to thank Michael James Stablein of the University of Illinois Urbana-Champaign for his translation services and review of this work.

The authors would also like to thank the Fundação de Amparo à Pesquisa do Estado de São Paulo for the fellowship provide to the PhD student Volnei Brito de Souza (Process #13/09090-2), and the financial support provided (Process #13/24874-0). Favaro-Trindade C.S. thanks the Brazilian National Council for Scientific and Technological Development (CNPq) for the productivity grant (#306708/2015-9).

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.foodhyd.2017.09.040.

References

Alvim, I. D., & Grosso, C. R. F. (2010). Microparticles obtained by complex coacervation: Influence of the type of reticulation and the drying process on the release of the core material. *Ciência e Tecnologia de Alimentos, 30*(4), 1069–1076.

Anderson, R. A., Broadhurst, C. L., Polansky, M. M., Schmidt, W. F., Khan, A., Flanagan, V. P., et al. (2004). Isolation and characterization of polyphenol type-A polymers from cinnamon with insulin-like biological activity. *Journal of Agricultural and Food Chemistry, 52*, 65–70.

Anhê, F. F. (2013). Polyphenols and type 2 diabetes: A prospective review. *Pharma Nutrition, 1*, 105–114.

Anvari, M., & Chung, D. (2016). Dynamic rheological and structural characterization of fish gelatina-Gum Arabic coacervate gels cross-linked by tannic acid. *Food Hydrocolloids, 60*, 516–524.

Arshady, R. (1993). Microcapsules for food. *Journal of Microencapsulation, 10*(4), 413–435.

Beecher, G. R. (2004). Proanthocyanidins: Biological activities associated with human health. *Pharmaceutical Biology, 42*(Supplement), 2–20.

Bennick, A. (2002). Interaction of plant polyphenols with salivary proteins. *Critical Reviews in Oral Biology & Medicine, 13*(2), 184–196.

Benzie, I. F. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: The FRAP assay. *Analytical Biochemistry, 239*, 70–76.

Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT – Food Science and Technology, 28*(1), 25–31, 1995.

Clinical and Laboratory Standards Institute – CLSI. (2009a). *Performance standards for antimicrobial disk susceptibility tests*. Approved Standard M02-A10 (10th ed.). Wayne: Clinical and Laboratory Standards Institute.

Clinical and Laboratory Standards Institute – CLSI. (2009b). *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically*. Approved Standard M7-A8 (8th ed.). Wayne: Clinical and Laboratory Standards Institute.

Comunian, T. A., Gomez-Estaca, J., Ferro-Furtado, R., Andrade Conceição, G. J., Freitas Moraes, I. C., Castro, I. A., et al. (2016). Effect of different polysaccharides and crosslinkers on echium oil microcapsules. *Carbohydrate Polymers, 150*, 319–329.

Comunian, T. A., Thomazini, M., Alves, A. J. G., de Matos Junior, F. E., de Carvalho Balieiro, J. C., & Favaro-Trindade, C. S. (2013). Microencapsulation of ascorbic acid by complex coacervation: Protection and controlled release. *Food Research International, 52*(1), 373–379.

Dai, R., Wu, G., Li, W., Zhou, Q., Li, X., & Chen, H. (2010). Gelatin/carboxymethylcellulose/dioctyl sulfosuccinate sodium microcapsule by complex coacervation and its application for electrophoretic display. *Colloids and Surfaces A: Physicochemical and Engineering Aspects, 362*, 84–89.

Edelmann, A., Diewok, J., Schuster, K. C., & Lendl, B. (2001). Rapid method for the discrimination of red wine cultivars based on mid-infrared spectroscopy of phenolic wine extracts. *Journal of Agricultural and Food Chemistry, 49*, 1139–1145.

Fávaro-Trindade, C. S., Pinho, S. C., & Rocha, G. A. (2008). Revisão: Microencapsulação de ingredientes alimentícios. *Brazilian Journal of Food Technology, 11*(2), 103–112.

Fujita, A., Borges, K., Correia, R., Franco, B. D. G. M., & Genovese, M. I. (2013). Impact of spouted bed drying on bioactive compounds, antimicrobial and antioxidant activities of commercial frozen pulp of camu-camu (*Myrciaria dubia* Mc. Vaugh). *Food Research International, 54*, 495–500.

García-Saldaña, J. S., Campas-Baypoli, O. N., López-Cervantes, J., Sánchez-Machado, D. I., Cantú-Soto, E. U., & Rodríguez-Ramírez, R. (2016). Microencapsulation of sulforaphane from broccoli seed extracts by gelatin/gum Arabic and gelatin/pectin complexes. *Food Chemistry, 201*, 94–100.

Gomez-Estaca, J., Comunian, T. A., Montero, P., Ferro-Furtado, R., & Favaro-Trindade, C. S. (2016). Encapsulation of an astaxanthin-containing lipid extract from shrimp waste by complex coacervation using a novel gelatin-cashew gum complex. *Food Hydrocolloids, 61*, 155–162.

Gruenwald, J., Freder, J., & Armbruester, N. (2010). Cinnamon and health. *Critical Reviews in Food Science and Nutrition, 50*, 822–834.

Hofmann, T., Glabasnia, A., Schwarz, B., Wisman, K. N., Gangwer, K. A., & Hagerman, A. E. (2006). Protein binding and astringent taste of a polymeric procyanidin, 1,2,3,4,6-penta-*O*-galloyl-b-D-glucopyranose, castalagin, and grandinin. *Journal of Agricultural and Food Chemistry, 54*, 9503–9509.

Ho, C. T., Rafi, M. M., & Ghai, G. (2010). Substâncias bioativas: Nutracêuticas e tóxicas. In S. Damodaran, K. L. Parkin, & O. Fennema (Eds.), *R. Química de alimentos de Fennema* (4. Ed., pp. 585–609). Porto Alegre: Artmed.

Ilic, I., Dreu, R., Burjak, M., Homar, M., Kerc, J., & Srci, S. (2009). Microparticle size control and glimepiride microencapsulation using spray congealing technology. *International Journal of Pharmaceutics, 381*, 176–183.

Jiao, L., Zhang, X., Huang, L., Gong, H., Cheng, B., Sun, Y., et al. (2013). Proanthocyanidins are the major anti-diabetic components of cinnamon water extract. *Food and Chemical Toxicology, 56*, 398–405.

Khan, A., Safdar, M., Ali-Khan, M. M., Khattak, K. N., & Anderson, R. A. (2003). Cinnamon improves glucose and lipids of people with type 2 diabetes. *Diabetes Care, 26*, 3215–3218.

De Kruif, C. G., Weinbreck, F., & De Vries, R. (2004). Complex coacervation of proteins and anionic polysaccharides. *Current Opinion in Colloid & Interface Science, 9*, 340–349.

Lv, Y., Zhang, X., Abbas, S., & Karangwa, E. (2012). Simplified optimization for microcapsule preparation by complex coacervation based on the correlation between coacervates and the corresponding microcapsule. *Journal of Food Engineering, 111*, 225–233.

Marcomini, R. F., & Souza, D. M. P. F. (2011). Caracterização microestrutural de materiais cerâmicos utilizando o programa de processamento digital de imagens. *Image J. Cerâmica, 57*, 100–105.

Mccue, P. P., Kwon, Y.-I., & Shetty, K. (2005). Anti-amylase, anti-glucosidase and anti-angiostension I converting enzyme potential of selected foods. *Journal of Food Biochemistry, 29*, 278–294.

Munin, A., & Edwards-Lévy, F. (2011). Encapsulation of natural poly-phenolic compounds: A review. *Pharmaceutics, 3*(4), 793–829.

Nonaka, G.-I., Morimoto, S., & Nishioka, I. (1983). Tannins and related compounds. Part 13.[1] isolation and structures of trimeric, tetrameric, and pentameric proanthicyanidins from cinnamon. *Journal of Chemical Society, Perkin Transactions 1, 1*, 2139–2145.

Nori, M. P., Favaro-Trindade, C. S., Matias de Alencar, S., Thomazini, M., de Camargo Balieiro, J. C., & Contreras Castillo, C. J. (2011). Microencapsulation of propolis extract by complex coacervation. *LWT - Food Science and Technology, 44*(2), 429–435.

Oehme, A., Valotis, A., Krammer, G., Zimmermann, I., & Schreier, P. (2011). Preparation and characterization of shellac-coated anthocyanin pectin beads as dietary colonic delivery system. *Molecular Nutrition & Food Research, 55*, S75–S85.

Payne, M. J., Hurst, W. J., Stuart, D. A., Ou, B., Fan, E., Ji, H., et al. (2010). Determination of total procyanidins in selected chocolate and confectionery products using DMAC. *Journal of AOAC International, 93*(1), 89–96.

Ping, L., Pizzi, A., Guo, Z. D., & Brosse, N. (2012). Condensed tannins from grape pomace: Characterization by FTIR and MALDI TOF and production of

environment friendly wood adhesive. *Industrial Crops and Products, 40*, 13–20.
Rocha-Selmi, G. A., Bozza, F. T., Thomazini, M., Bolini, H. M. A., & Fávaro-Trindade, C. S. (2013b). Microencapsulation of aspartame by double emulsion followed by complex coacervation to provide protection and prolong sweetness. *Food Chemistry, 139*, 72–78.
Rocha-Selmi, G. A., Theodoro, A. C., Thomazini, M., Bolini, H. M. A., & Favaro-Trindade, C. S. (2013a). Double emulsion stage prior to complex coacervation process for microencapsulation of sweetener sucralose. *Journal of Food Engineering, 119*, 28–32.
Rohr, G. E., Meier, B., & Sticher, O. (2000). Analysis of procyanidins. In A. Rahman (Ed.), *Studies in natural products chemistry* (Vol. 21, pp. 497–570). Elsevier Science B.V.
Santiago-Adame, R., Medina-Torres, L., Gallegos-Infante, J. A., Calderas, F., González-Laredo, R. F., Rocha-Guzmán, N. E., et al. (2015). Spray drying-microencapsulation of cinnamon infusions (*Cinnamomum zeylanicum*) with maltodextrin. *LWT - Food Science and Technology, 64*, 571–577.
Santos, M. G., Bozza, F. T., Thomazini, M., & Favaro-Trindade, C. S. (2015). Microencapsulation of xylitol by double emulsion followed by complex coacervation. *Food Chemistry, 171*, 32–39.
Schmitt, C., & Turgeon, S. L. (2011). Protein/polysaccharide complexes and coacervates in food systems. *Advances in Colloid and Interface Science, 167*, 63–70.
Shan, B., Cai, Y. Z., Brooks, J. D., & Corke, H. (2007). Antibacterial properties and major bioactive components of cinnamon stick (*Cinnamomum burmanii*): Activity against foodborne pathogenic bacteria. *Journal of Agricultural and Food Chemistry, 55*, 5484–5490.
Silva, D. F., Favaro-Trindade, C. S., Rocha, G. A., & Thomazini, M. (2012). Microencapsulation of lycopene by gelatin–pectin complex coacervation. *Journal of Food Processing and Preservation, 36*, 185–190.
Singh, O. N., & Burgess, D. J. (1989). Characterisation of albumin-alginic acid complex coacervation. *Journal of Pharmacy and Pharmacology, 41*, 670–673.
Singleton, V. L., Orthofer, R., & Lamuela-Raventos, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods in Enzymology, 299*, 178–184.
Sitthichai, S., Pilapong, C., Thongtem, T., & Thongtem, S. (2015). CMC-coated Fe_3O_4 nanoparticles as new MRI probes for hepatocellular carcinoma. *Applied Surface Science, 356*, 972–977.
Souza, V. B., Thomazini, M., Balieiro, J. C. C., & Favaro Trindade, C. S. (2015). Effect of spray drying on the physicochemical properties and color stability of the powdered pigment obtained from vinification byproducts of the Bordo grape (*Vitis labrusca*). *Food and Bioproducts Processing, 93*, 39–50.
Souza, V. B., Thomazini, M., Petta, T., Tulini, F. L., Oliveira, C. A. F., Genovese, M. I., et al. (2017). *Obtaining of a rich proanthocyanidins extract from Ceylon cinnamon (Cinnamomum zeylanicum): Extraction kinetics, identification of the main fractions and antioxidant capacity*. Unpublished results.
Strauss, G., & Gibson, S. M. (2004). Plant phenolics as cross-linkers of gelatin gels and gelatin-based coacervates for use as food ingredients. *Food Hydrocolloids, 18*, 81–89.
Timilsena, Y. P., Adhikarib, R., Barrow, C. J., & Adhikari, B. (2016). Microencapsulation of chia seed oil using chia seed protein isolate-chia seed gum complex coacervates. *International Journal of Biological Macromolecules, 91*, 347–357.
Trinh, T. H., Shaari, K. Z. K., Basit, A., & Azeem, B. (2014). Effect of particle size and coating thickness on the release of urea using multi-diffusion model. *International Journal of Chemical Engineering and Applications, 5*(1), 58–63.
Tulini, F. L., Souza, V. B., Echalar-Barrientos, M. A., Thomazini, M., Pallone, E. M. J. A., & Fávaro-Trindade, C. S. (2016). Development of solid lipid microparticles loaded with a proanthocyanidin-rich cinnamon extract (*Cinnamomum zeylanicum*): Potential for increasing antioxidant content in functional foods for diabetic population. *Food Research International, 85*, 10–18.
Tulini, F. L., Souza, V. B., Thomazini, M., Silva, M. P., Massarioli, A. P., Alencar, S. M., et al. (2017). Evaluation of the release profile, stability and antioxidant activity of a proanthocyanidin-rich cinnamon (*Cinnamomum zeylanicum*) extract co-encapsulated with α-tocopherol by spray chilling. *Food Research International, 95*, 117–124.
Worthington, V. (Ed.). (1993). *Alpha amylase. Worthington enzyme manual* (pp. 36–41). Freehold, NJ: Worthington Biochemical.