International Journal of Food Science +Technology

International Journal of Food Science and Technology 2022, **57**, 145–153

Original article

Microencapsulation of roasted coffee oil Pickering emulsions using spray- and freeze-drying: physical, structural and *in vitro* bioaccessibility studies

Elisa Franco Ribeiro,[1,2*] Tiago Carregari Polachini,[1] Izabela Dutra Alvim,[3] Amparo Quiles,[2] Isabel Hernando[2] & Vania Regina Nicoletti[1]

1 São Paulo State University (Unesp), Institute of Biosciences, Humanities and Exact Sciences (Ibilce), Campus São José do Rio Preto, São Paulo 15054-000, Brazil

2 Food Microstructure and Chemistry Research Group, Universitat Politècnica de València (UPV), Valencia 46022, Spain

3 Cereal and Chocolate Technology Center, Food Technology Institute (ITAL), Campinas São Paulo, 13070-178, Brazil

(Received 5 August 2021; Accepted in revised form 28 September 2021)

Summary Microcapsules produced from well-defined emulsion templates are an interesting alternative for lipid encapsulation. This work aimed to produce microcapsules by the freeze-drying (FD) and spray-drying (SD) of Pickering emulsions of roasted coffee oil (RCO) stabilised with chitosan nanoparticles produced by self-aggregation or by crosslinking with tripolyphosphate. The dried microcapsules were characterised in terms of particle size, oil retention and structure; furthermore, the *in vitro* bioaccessibility of polyphenols from microencapsulated RCO was investigated. The use of chitosan nanoparticles to stabilise the emulsions increased oil retention in the microcapsules giving values between 83.04% and 95.36%. SD produced spherical microcapsules with small particle sizes (~11 μm), whereas FD microcapsules showed an irregular shape and porous structure. Although FD had the lowest impact on the bioactive compounds, SD promoted better protection for phenolic compounds and antioxidant activity during *in vitro* digestion.

Keywords Antioxidant activity, bioactive compounds, chitosan nanoparticles, *in vitro* digestion, lipid encapsulation, phenolic content.

Highlights

- Freeze-drying better preserves bioactive compounds
- Spray-drying showed better bioaccessibility of phenolic compounds
- Chitosan played an important role in the production of microcapsules
- Microencapsulation improved oil retention in both drying methods
- Spray-drying technology should be improved to give higher drying yields

Introduction

The stabilisation of oil-in-water emulsions using the Pickering method has been strongly encouraged as an alternative to replace the conventional surfactants with natural compounds such as biopolymers. These macromolecules may be converted into colloidal particles that can adsorb onto the oil/water interface, thus forming a robust monolayer that prevents droplet coalescence and stabilises the emulsions because of the high energy level required to remove them from the interface (Yan *et al.*, 2017). To optimise the performance of biopolymers as Pickering emulsifiers, physical and/or chemical modifications must be carried out to make them suitable for a given application (Jiang *et al.*, 2020; Li *et al.*, 2021).

Chitosan is a chitin-derived polysaccharide with particularly high biocompatibility and biodegradability and has been used to replace petrochemical-synthesised polymers (Pérez-Guzmán & Castro-Muñoz, 2020). Due to their good film-forming properties (Díaz-Montes & Castro-Muñoz, 2021), chitosan has been studied as a selective barrier for membrane-based separation process (Castro-Muñoz *et al.*, 2020) and also to control the delivery of bioactive compounds through encapsulation (Carlan *et al.*, 2017). This polysaccharide presents high solubility in an acid

*Correspondent: E-mail: mihernan@tal.upv.es

doi:10.1111/ijfs.15378

medium due to the protonation phenomena. However, as the electric charges of chitosan are neutralised, it becomes more hydrophobic, assuming a specific conformational structure desirable for food purposes (Baek *et al*., 2019). Among the many methods for developing colloidal chitosan particles, the self-aggregation and crosslinking techniques are widely reported in the literature – including for Pickering emulsification (Mwangi *et al*., 2016; Costa *et al*., 2018; Ribeiro *et al*., 2020a,b). Self-aggregation is performed by deprotonating chitosan molecules in the presence of an alkaline agent, causing the molecules to self-assemble into solid micro- or nanoparticles (Ho *et al*., 2016). Crosslinking is an alternative method to produce chitosan nanoparticles that comprise adding a crosslinking agent, for example, sodium tripolyphosphate (TPP), which results in the ionic gelation of chitosan chains through the interaction between the negative charges of the polyanion and the positive charges of the primary amino groups present in chitosan molecules. The formation of chitosan nanoparticles following these two approaches was explored in a previous work (Ribeiro *et al*., 2020a), in which both nanoparticles were characterised regarding their physicochemical properties, as well as their performance in the stabilisation of Pickering emulsions.

Emulsions play an important role in preserving lipid compounds (Araiza-Calahorra *et al*., 2018), but their handling and shelf-life are impaired because of their high water content. Thus, converting emulsions into powdered formulations by removing water is an attractive possibility for microencapsulation of food compounds, in addition to reducing costs related to storage and transportation. Freeze-drying (FD) and spray-drying (SD) are some of the most used methods for encapsulating materials of interest in a food matrix.

The different mechanisms involved in drying and encapsulation by FD and SD give rise to dried microcapsules with distinct composition, microstructure and physical properties that could affect the delivery of bioactive compounds in the gastrointestinal tract (Karthik & Anandharamakrishnan, 2013; Mwangi *et al*., 2016). Studies about bioaccessibility have been successfully carried out to evaluate the release of antioxidants and phenolic compounds from the lipid core during simulated *in vitro* digestion (Jara-Palacios *et al*., 2018; Burgos-Díaz *et al*., 2020), including from Pickering emulsions stabilised by chitosan nanoparticles (Ribeiro *et al*., 2020b). On the other hand, there is little information about the effects of different drying methods on the bioaccessibility of compounds microencapsulated by drying Pickering emulsions.

In this study, roasted coffee oil (RCO) was the lipid phase to be encapsulated. RCO is a coproduct of the coffee processing industry and presents high contents of unsaturated fatty acids and volatile compounds (Oliveira *et al*., 2005; Hurtado-Benavides *et al*., 2016; Ribeiro *et al*., 2020b). Moreover, the presence of caffeine and chlorogenic acids as well as tocopherols and diterpenes has been responsible for a high antioxidant capacity (Böger *et al*., 2021). Encapsulating RCO has been reported as an efficient way to protect the important compounds of oil (Freiberger *et al*., 2015; Zanin *et al*., 2021). However, little information is reported about the mechanisms of microcapsules' synthesis and their role in the release of bioactive compounds.

Unlike conventional emulsions commonly used for encapsulation, Pickering emulsions stabilised by self-aggregated or crosslinked chitosan nanoparticles, which have been previously characterised by Ribeiro *et al*. (2020a), were used in this study as templates for producing dried microcapsules by means of two different drying methods: FD and SD. The particle size distribution and microstructure of the dried microcapsules were studied, as well as the effects of the different chitosan nanoparticles and different drying methods on the bioaccessibility of the bioactive compounds contained in the encapsulated RCO.

Material and methods

Materials

The following ingredients were used in this study: low molecular weight chitosan powder (degree of deacetylation: 77%) was purchased from Sigma-Aldrich (Saint Louis, USA); roasted coffee oil (RCO) was kindly supplied by Cia. Iguaçu de Café Solúvel (Cornélio Procópio, Brazil); maltodextrin DE 10 was purchased from Get do Brasil (São João da Boa Vista, Brazil); sodium tripolyphosphate (TPP) was purchased from LS Chemicals (Ribeirão Preto, Brazil); glacial acetic acid and sodium hydroxide were purchased from Dinâmica (Indaiatuba, Brazil). Analytical grade chemicals and ultrapure water with 18.2 MΩ cm resistivity were used in all the experiments.

Synthesis of chitosan nanoparticles

Chitosan nanoparticles were obtained using the two methods as described by Ribeiro *et al*. (2020a). Self-aggregated chitosan nanoparticles were produced by deprotonation of the amino groups, whereas crosslinked chitosan nanoparticles were obtained by adding sodium tripolyphosphate (TPP) as a crosslinking agent. For the deprotonation method, nanoparticles were obtained by dripping 6 M NaOH to increase the pH of the solution to 6.7. For the crosslinking method, a TPP aqueous solution was added to the initial chitosan solution (CS) to give the mass ratio of 3: 1 (CS: TPP). Both methods aimed at obtaining a final suspension concentration of chitosan nanoparticles of 0.9 g 100 g^{-1}.

Preparation of RCO Pickering emulsions

Roasted coffee oil (10 g) was added under homogenisation (Ultra-Turrax T25, IKA, Germany) at 2900 g to the suspensions (90 g) of self-aggregated or crosslinked chitosan nanoparticles to produce emulsions with 10% (w/w) of the lipid phase. After the oil was added, the samples were mixed for 5 min. Both emulsions were prepared in triplicate.

Production of microcapsules

Microcapsules were produced starting from the RCO emulsions prepared as described in Section 2.3 added with maltodextrin DE 10 (35 g 100 g^{-1} of emulsion), which was used as a carrier agent. Maltodextrin was dispersed in the emulsions using an Ultra-Turrax homogeniser at 12 000 rpm for 5 min. As a control treatment, RCO was dispersed directly in a solution of maltodextrin (35 g 100 g^{-1}) in ultrapure water without chitosan nanoparticles, resulting in the treatment coded as MO. Maltodextrin-added emulsions containing self-aggregated chitosan were denominated as CMO, whereas those with crosslinked chitosan were designated as CTMO. After preparation, the control treatment and the maltodextrin-added emulsions were subjected to FD or SD.

Freeze-drying (FD)
For FD, the emulsions were frozen at −40 °C in an ultra-freezer for 24 h, followed by drying by water sublimation in a freeze-dryer (model L-101, Liotop, Brazil) for 48 h at an approximate pressure of 200 μmHg. Samples were then ground manually using a pestle and a mortar and stored in metallised bags in a desiccator at room temperature. This procedure produced the FD microcapsules MO-FD (control), CMO-FD and CTMO-FD.

Spray-drying (SD)
For the SD process, the emulsions were dried in a bench scale spray dryer (B-290, Büchi, Switzerland) using a double-fluid spray nozzle (orifice diameter = 0.7 mm). The equipment was fed through a peristaltic pump and, after preliminary assays, the defined drying conditions were: feed flow rate of 2 mL min^{-1}, atomisation air rate of 742 L h^{-1}, inlet air temperature 160 °C and aspiration rate of 35 m^3 h^{-1}. The SD samples were designated as MO-SD (control) and CMO-SD (formulated with self-aggregated chitosan) and were stored in metallised bags in a desiccator at room temperature. SD microcapsules obtained from the emulsion containing TPP-crosslinked nanoparticles could not be produced because the TPP-chitosan nanoparticles presented a very rigid structure (Ribeiro *et al*., 2020a), and their presence in CTMO emulsions caused the nozzle feeding orifice to clog as the sample was pumped.

Characterisation of microcapsules

Particle size distribution
Particle size distribution of SD and FD microcapsules was measured using laser diffraction (L950, Horiba Instruments, Inc., Japan). The microcapsules were dispersed into ethanol at concentrations adjusted to reach the transmittance levels required by the equipment to perform the reading. The mean particle size, expressed as the volume mean diameter $D[4,3]$, and the width of the particle size distribution (*Span*) were calculated according to eqns (1) and (2) respectively:

$$D[4,\ 3] = \frac{\sum n_i d_i^4}{\sum n_i d_i^3} \tag{1}$$

$$\text{Span} = \frac{D_{90} - D_{10}}{D_{50}} \tag{2}$$

where n_i is the number of particles with d_i diameter. D_{10} is the diameter at which 10% of the particles lie below this value. D_{50} and D_{90} correspond to the diameters at which 50% and 90% of the cumulative volumes of the distribution have smaller particle sizes than that value respectively.

Oil retention
Roasted coffee oil retention (%) in the microcapsules was determined using a Multiskan Go Spectrophotometer (Thermo Fisher Scientific, Vantaa, Finland) following the methodology proposed by Freiberger *et al*. (2015) with some modifications. The microcapsules (0.5 g) were dissolved in 1% acetic acid solution (5 mL) while stirring for 10 min. Chitosan was precipitated by adding ethanol (5 mL), then the solution was filtered through a 0.45-μm nylon filter and the absorbance was read at 296 nm. As a blank, used for absorbance readings, a suspension of microcapsules produced without RCO was subjected to the same treatment. A calibration curve was previously obtained for calculating RCO concentration. The oil retention was obtained using eqn (3):

$$\text{Retention}(\%) = 100 \times \frac{C_t}{C_0} \tag{3}$$

where C_t is the total oil content in the microcapsules (g) and C_0 is the theoretical oil content (g) expected in the powder, based on the emulsion formulation on a dry basis.

Drying yield
The drying yield (%) corresponding to each drying method was determined as the ratio between the total

solids recovered and the mass of solids present in the dried emulsions.

Microstructure
The microstructure of SD and FD samples was analysed using scanning electron microscopy. Samples were coated with platinum and observed by a field emission scanning electron microscope (FESEM) (Ultra55 FESEM model; Zeiss, Oberkochen, Germany). Each sample was analysed in duplicate.

Total phenolic content and antioxidant capacity
The total phenolic content (TPC) was determined in triplicate using the Folin–Ciocâlteu assay (Singleton & Rossi, 1965) and the ferric-reducing antioxidant power (FRAP) was evaluated following Benzie & Strain (1996). For both methods, ethanolic extracts were obtained by stirring the samples with absolute ethanol (10 mg mL^{-1}) for 10 s in a vortex and centrifuging at 19400 g for 20 min at 4 °C (Ribeiro *et al*., 2020b). The content of TPC was expressed as mg of gallic acid equivalents (GAE) 100 g^{-1} of dry matter and the antioxidant activity was expressed as μmol Trolox g^{-1} (dry matter) of sample.

In vitro digestion

In vitro digestion model
A simulated *in vitro* digestion, following the methodology described by Eriksen *et al*. (2017), Gómez-Mascaraque *et al*. (2017) and Minekus *et al*. (2014), was performed to evaluate the effects of chitosan nanoparticle synthesis and drying methods in the bioaccessibility of the phenolic and antioxidant compounds present in the microcapsules. The digestion process was carried out in a reaction station Carousel 6 Plus with a controlled temperature of 37 °C and stirring at 150 rpm. During digestion, the samples were kept in the dark and in nitrogen atmosphere in both the gastric and intestinal phases. Solutions of simulated salivary fluid (SSF), simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) were prepared according to the compositions described by Minekus *et al*. (2014). At the end of the digestion, the resulting mixture was centrifuged at 14 500 rpm for 20 min at 4 °C) and filtered using a vacuum pump and Whatman no.4 filter paper, 20–25-μm pore size, yielding a two-phase system. The supernatant, considered to present the soluble compounds available to be transferred to the human blood, was designed as *IN* fraction and it was used for calculating the bioaccessibility of total phenolic compounds and antioxidant capacity.

Bioaccessibility
The phenolic content of RCO and its antioxidant capacity were used to determine the bioaccessibility index as suggested by Schulz *et al*. (2017) with some modifications.

The bioaccessibility index is defined as the proportion of the TPC or antioxidant capacity present in the intestinal phase that could become available for absorption into the systemic circulation. It was calculated according to eqn (4):

$$\text{Bioaccessibility index}(\%) = \frac{\text{IN}}{\text{ND}} \times 100 \qquad (4)$$

where IN is the content of the compound released in the simulated digestion and ND is the content of the compound in the non-digested sample.

Statistical analysis

Analysis of variance (ANOVA) was performed on the experimental data using the STATISTICA software (StatSoft Inc., Tulsa, OK, USA). Significant differences between the averages was evaluated by the Fisher's test with 95% of confidence.

Results and discussion

Characterisation of microcapsules

Particle size distribution
As expected, SD resulted in significantly ($P < 0.05$) smaller particles than FD because of the atomisation of small emulsion droplets followed by hot air drying (Table 1). Besides, the absence of significant differences among the *D*[4,3] values between SD samples meant that using self-aggregated chitosan for the encapsulation did not cause significant changes in the particle size of microcapsules.

Table 1 Particle size measurements of RCO microcapsules produced from chitosan-based Pickering emulsions by different drying methods

Sample	*D*[4,3]	*Span*
MO-FD	276.21 ± 48.18^{a}	3.004 ± 0.183^{b}
CMO-FD	244.45 ± 41.39^{b}	2.942 ± 0.198^{b}
CTMO-FD	231.91 ± 24.80^{b}	3.371 ± 0.342^{a}
MO-SD	10.73 ± 0.22^{c}	1.305 ± 0.006^{d}
CMO-SD	12.83 ± 0.20^{c}	1.698 ± 0.016^{c}

MO-FD = freeze-dried microcapsules of RCO with maltodextrin. CMO-FD = freeze-dried microcapsules of RCO with self-aggregated chitosan and maltodextrin. CTMO-FD = freeze-dried microcapsules of RCO with TPP-crosslinked chitosan and maltodextrin. MO-SD = spray-dried microcapsules of RCO with maltodextrin. CMO-SD = spray-dried microcapsules of RCO with self-aggregated chitosan and maltodextrin. Data are expressed as means values ± standard deviation. Values with different letters within the same column are significantly different ($P < 0.05$) according to the LSD (Fisher's) multiple range test.

Regarding the FD samples, they resulted in similar particle sizes ($P > 0.05$)–between CMO-FD and CTMO-FD–with a higher polydispersity (higher *Span* values) than those obtained by SD, as they were manually milled, giving rise to heterogeneous particles; this was also determined by Šturm *et al.* (2019) for FD propolis. Significantly higher values ($P < 0.05$) were noticed for the control sample, which is probably related to an impaired encapsulation by the exclusive use of maltodextrin as a carrier agent that may have led to clump formation due to free oil on the surface. Adding chitosan nanoparticles plays the role of creating a better interfacial film that prevents the resulting microcapsules from agglomerating (Li *et al.*, 2018). The good interaction between coffee oil and matrix was previously described by Ribeiro *et al.* (2020). Figure 1 presents the particle size distribution for the studied samples. In this figure, higher particle size and polydispersity with a single large peak could be observed for samples obtained by FD. SD produced microcapsules with a smaller size, but the MO-SD sample showed two peaks in contrast to the single peak observed for CMO-SD.

Although two peaks show interesting packing and transport purposes, due to better penetration of smaller particles into the spaces between those larger (Silva *et al.*, 2014), the oil retention should be carefully analysed to ensure the microcapsules could encapsulate the lipid phase adequately.

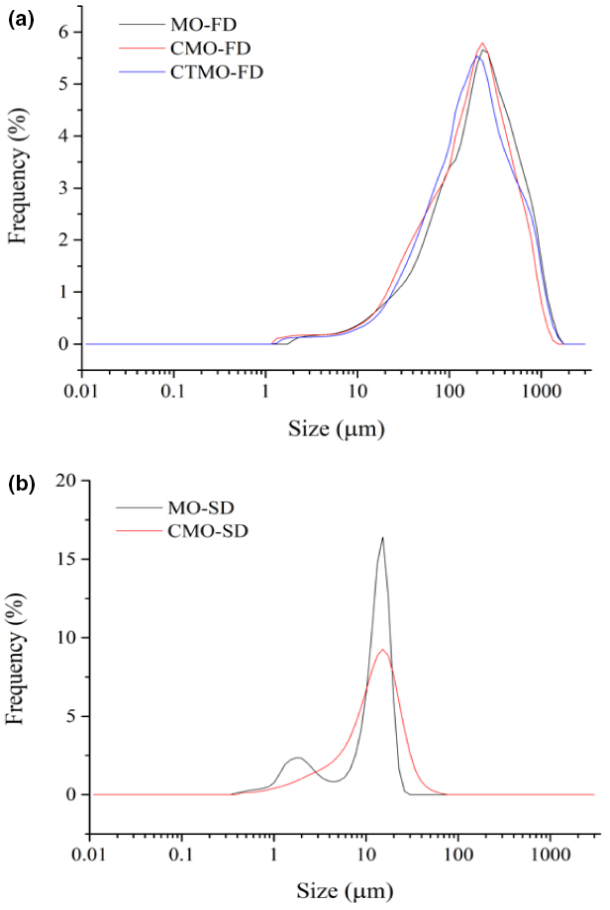

Figure 1 Particle size distribution of RCO microcapsules produced from chitosan-based Pickering emulsions by (a) freeze-drying (FD) and (b) spray-drying (SD). CMO, self-aggregated chitosan and maltodextrin; CTMO, crosslinked chitosan and maltodextrin; MO, only maltodextrin.

Drying yield and oil retention

As expected, FD reached the highest values of drying yield–close to 100% (Table 2). SD yielded lower amounts of powdered material, mainly due to common adhesion losses in the drying chamber wall.

Regarding oil retention for FD samples, a substantial part of the lipid phase was retained in the microparticles (76%–95%); however, lower oil retention was observed for the control samples. For SD samples, CMO-SD presented a significantly ($P < 0.05$) higher value for oil retention. The oil retention values observed are close to the values found by Francisco *et al.* (2020) on the microencapsulation of orange essential oil using soy protein isolate (SPI) and pea protein concentrate (PPC). Authors attributed the higher oil retention values observed (92.6%–97.9%) to the better stability of SPI emulsions during the atomisation process. Alcântara *et al.*, (2019) also reported the importance of the wall material and the homogenisation process for producing stable chia microparticles. For both drying methods used in the present work, chitosan nanoparticles improved the oil retention capacity in the microcapsules probably due to the high emulsification capacity, preventing droplet coalescence

Table 2 Drying yield and oil retention of RCO microcapsules

Sample	Drying yield (%)	Oil retention (%)
MO-FD	89.95 ± 2.56^{a}	76.29 ± 11.41^{b}
CMO-FD	92.69 ± 0.13^{a}	85.15 ± 2.35^{ab}
CTMO-FD	91.16 ± 1.38^{a}	95.36 ± 6.26^{a}
MO-SD	19.16 ± 2.56^{c}	40.18 ± 6.39^{c}
CMO-SD	25.47 ± 0.70^{b}	83.04 ± 8.94^{ab}

MO-FD = freeze-dried microcapsules of RCO with maltodextrin. CMO-FD = freeze-dried microcapsules of RCO with self-aggregated chitosan and maltodextrin. CTMO-FD = freeze-dried microcapsules of RCO with TPP-crosslinked chitosan and maltodextrin. MO-SD = spray-dried microcapsules of RCO with maltodextrin. CMO-SD = spray-dried microcapsules of RCO with self-aggregated chitosan and maltodextrin. Data are expressed as mean values ± standard deviation. Values with different letters within the same column are significantly different ($P < 0.05$) according to the LSD (Fisher's) multiple range test.

and avoiding the deposition of non-encapsulated oil on the surface of the powders (Taboada *et al*., 2021).

Microstructure

The electronic micrographs showed SD produced more spherical microcapsules without the presence of apparent fissures (Figure 2), potentially preventing the core oxidation due to the probably lower permeability to oxygen and moisture (Kumar *et al*., 2017). The observed wrinkled surface was expected due to the rapid water loss during SD (Fang *et al*., 2019).

Although the two spray-dried samples demonstrated similar morphology, the MO samples showed a noticeable number of particles smaller than 4 μm around those bigger ones, with a particle size of ≈15 μm. This morphology closely agrees with the bimodal particle size distribution in Fig. 1b. The poor stabilisation of emulsions containing exclusively maltodextrin as a carrier agent probably led to lower oil retention (Table 2) and the possible production of empty capsules of smaller sizes. By producing smaller particles, the area/volume ratio of SD samples was higher than those of FD.

Freeze-drying the RCO emulsions resulted in microparticles with irregular shapes and higher particle size than SD samples, as observed in Fig. 2. These microparticles seemed more porous (white arrows) than SD due to the sublimation of the water crystals formed between the oil droplets during freezing. Besides, the micrographs showed these microparticles presented oil as an entrapped phase into the polymeric network.

The morphology of the microparticles obtained by SD or FD affects the capacity of the solid matrix, for protecting the encapsulated compounds during storage and the human gastrointestinal digestion (Burgos-Díaz *et al*., 2020). Therefore, the bioaccessibility of phenolic compounds and antioxidant capacity was studied, as shown in section 3.3.

Total phenolic contents and antioxidant capacity

Spray-dried samples showed significantly lower contents of phenolic compounds and antioxidant properties than the FD ones (Table 3). Furthermore, there were no significant differences in the total phenolic contents and antioxidant activity in SD samples formulated with or without self-aggregated chitosan. The lower TPC and antioxidant activity of SD samples are probably related to the specific structure of the

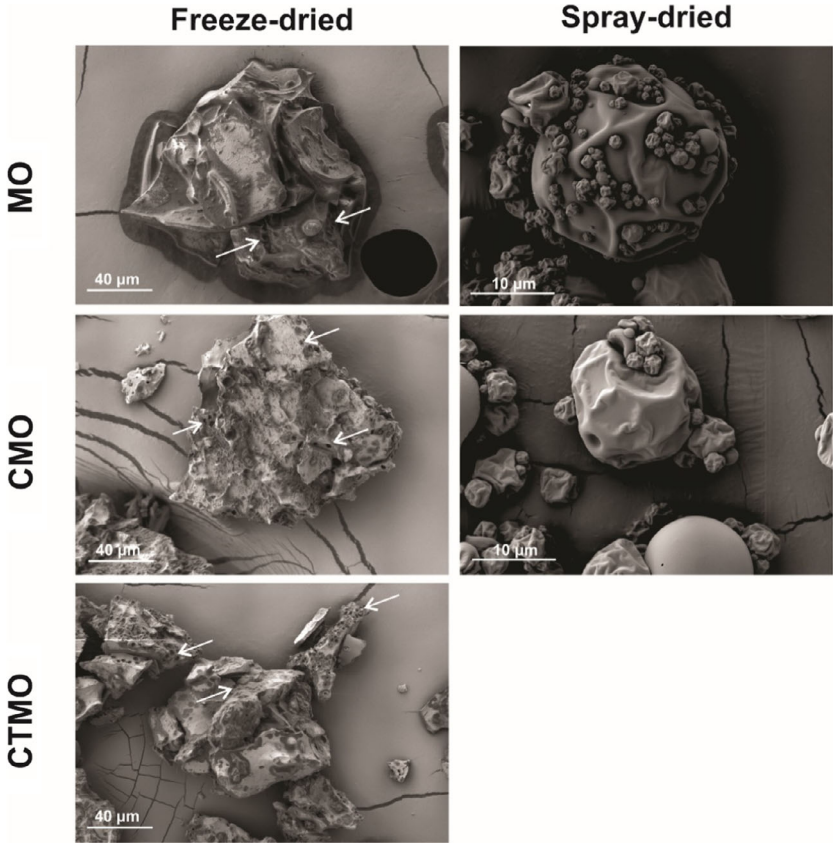

Figure 2 Field emission scanning electron microscopy for RCO microcapsules obtained by freeze-drying and spray-drying. CMO, self-aggregated chitosan and maltodextrin; CTMO, crosslinked chitosan and maltodextrin; MO, only maltodextrin.

Table 3 Total phenolic content (TCP) and antioxidant activity for freeze-dried and spray-dried samples

Sample	TPC (mg GAE 100 g^{-1} of dry matter)	FRAP (μmol Trolox g^{-1} of dry matter)
MO-FD	60.2 ± 0.2[a]	11.83 ± 0.82[a]
CMO-FD	46.3 ± 0.2[c]	5.08 ± 0.43[c]
CTMO-FD	50.4 ± 0.3[b]	8.18 ± 0.97[b]
MO-SD	18.5 ± 0.2[d]	3.19 ± 0.55[d]
CMO-SD	19.4 ± 0.2[d]	3.43 ± 0.56[d]

MO-FD = freeze-dried microcapsules of RCO with maltodextrin. CMO-FD = freeze-dried microcapsules of RCO with self-aggregated chitosan and maltodextrin. CTMO-FD = freeze-dried microcapsules of RCO with TPP-crosslinked chitosan and maltodextrin. MO-SD = spray-dried microcapsules of RCO with maltodextrin. CMO-SD = spray-dried microcapsules of RCO with self-aggregated chitosan and maltodextrin. Data are expressed as means values ± standard deviation. Values with different letters within the same column are significantly different ($P < 0.05$) according to the LSD (Fisher's) multiple range test.

Table 4 Bioaccessibility index (%) of the total phenolic content (TPC) and antioxidant activity for RCO microcapsules

	Bioaccessibility index	
	TPC (%)	FRAP (%)
MO-FD	243.75 ± 17.73[c]	132.50 ± 13.81[c]
CMO-FD	331.69 ± 1.25[b]	846.54 ± 37.22[b]
CTMO-FD	205.05 ± 12.88[c]	588.78 ± 81.52[b]
MO-SD	100.56 ± 21.10[d]	257.19 ± 63.10[c]
CMO-SD	548.77 ± 18.14[a]	1698.91 ± 246.76[a]

MO-FD = freeze-dried microcapsules of RCO with maltodextrin. CMO-FD = freeze-dried microcapsules of RCO with self-aggregated chitosan and maltodextrin. CTMO-FD = freeze-dried microcapsules of RCO with TPP-crosslinked chitosan and maltodextrin. MO-SD = spray-dried microcapsules of RCO with maltodextrin. CMO-SD = spray-dried microcapsules of RCO with self-aggregated chitosan and maltodextrin. Data are expressed as means values ± standard deviation. Values with different letters within the same column are significantly different ($P < 0.05$) according to the LSD (Fisher's) multiple range test.

spherical particles that protect the core, as observed in section 3.1.3.

Among the FD microparticles, the control samples formulated only with maltodextrin presented the highest TPC and antioxidant activity, followed by the ones produced with TPP-crosslinked chitosan and deprotonated chitosan. A likely explanation for this behaviour is that the porous microstructure of FD samples allows better radical scavenging by the antioxidant compounds entrapped in the matrix. As the oil phase was better retained by adding chitosan nanoparticles (CMO-FD and CTMO-FD samples), the TPC in the corresponding ethanolic extracts significantly decreased if compared to MO-FD. Comparing the CMO-FD and CMO-SD, which presented no significant differences in oil retention, a higher TPC and antioxidant activity were observed in CMO-FD, probably due to the low thermal degradation effects during FD (Rezvankhah *et al.*, 2020).

Bioaccessibility

Table 4 shows the bioaccessibility indexes for the studied samples.

Self-aggregated chitosan particles presented significantly higher ($P < 0.05$) bioaccessibility values both for SD and FD samples, even when comparing with TPP-crosslinked chitosan. In contrast to the crosslinked chitosan, self-aggregated nanoparticles can be reversely solubilised under acidic pH (Akbari-Alavijeh *et al.*, 2020), which can occur during the gastric step. This causes the Pickering particle to partially solubilise, and the material of interest is more easily released. Concerning the CTMO-FD samples, the release occurs mainly during the higher pH stages (intestinal phase), at which the ionisation of chitosan is lower and the ionic interactions between chitosan and TPP are weakened (Shu & Zhu, 2000). In this condition, the Pickering particle becomes more flexible to allow a mass transfer.

Altin *et al.*, (2018) reported that although low phenolic content was quantified before digestion due to the protection given by the encapsulation in SD chitosan liposomes, this value increased during a simulated gastrointestinal tract.

It can be assumed that the chitosan microcapsules produced by SD could act as an efficient barrier to promote a controlled release of bioactive compounds along digestion.

Conclusions

Spray-drying produced spherical microcapsules with a more homogeneous aspect and smaller particle size when compared to the freeze-dried microcapsules, which showed irregular shapes and porous structure. Using chitosan Pickering particles improved the oil retention with both drying methods. Although freeze-drying preserved the bioactive compounds due to the low temperature applied, the greatest bioaccessibility was obtained in spray-dried samples formulated with self-aggregated chitosan due to the high oil retention. However, it would be interesting to improve spray-drying technology, to consider the microcapsules' stability during storage for further applicability in food industries and to better study the behaviour release of RCO from the matrix.

Acknowledgments

The authors are thankful to the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior–CAPES (Finance Code 001; Grant number 88887.468140/2019-00), and to the São Paulo Research Foundation–FAPESP (Grant number 2016/22727-8).

Conflict of interest

All authors declare no conflict of interest.

Author contributions

Elisa Franco Ribeiro: Data curation (lead); Formal analysis (lead); Investigation (lead); Writing-original draft (lead). **Tiago Carregari Polachini:** Investigation (supporting); Writing-original draft (supporting). **Izabela Dutra Alvim:** Formal analysis (supporting); Methodology (supporting). **Amparo Quiles:** Methodology (equal); Resources (equal); Supervision (equal); Writing-review & editing (equal). **Isabel Hernando:** Methodology (equal); Resources (equal); Supervision (equal); Writing-review & editing (equal). **Vânia Regina Nicoletti:** Conceptualization (lead); Funding acquisition (lead); Resources (equal); Supervision (equal); Writing-review & editing (equal).

Ethical approval

Ethics approval was not required for this research.

Peer review

The peer review history for this article is available at https://publons.com/publon/10.1111/ijfs.15378.

Data availability statement

Research data are not shared.

References

Akbari-Alavijeh, S., Shaddel, R. & Jafari, S.M. (2020). Encapsulation of food bioactives and nutraceuticals by various chitosan-based nanocarriers. *Food Hydrocolloids*, **105**, 105774.

Alcântara, M.A., Lima, A.E.A.D., Braga, A.L.M. *et al.* (2019). Influence of the emulsion homogenization method on the stability of chia oil microencapsulated by spray drying. *Powder Technology*, **354**, 877–885.

Altin, G., Gültekin-Özgüven, M. & Ozcelik, B. (2018). Chitosan coated liposome dispersions loaded with cacao hull waste extract: Effect of spray drying on physico-chemical stability and in vitro bioaccessibility. *Journal of Food Engineering*, **223**, 91–98.

The authors reported the use of chitosan as a useful carrier agent to produce spray-dried microparticles containing bioactive compounds from cacao hull waste. They investigated how the chitosan coating in liposome dispersions affects the physico-chemical properties of the resulting spray-dried microparticles. Moreover, the authors also discussed about the role of chitosan in the *in vitro* digestion assays of such microparticles.

Araiza-Calahorra, A., Akhtar, M. & Sarkar, A. (2018). Recent advances in emulsion-based delivery approaches for curcumin: from encapsulation to bioaccessibility. *Trends in Food Science & Technology*, **71**, 155–169.

Baek, J., Wahid-Pedro, F., Kim, K. *et al.* (2019). Phosphorylated-CNC/modified-chitosan nanocomplexes for the stabilization of Pickering emulsions. *Carbohydrate Polymers*, **206**, 520–527.

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**(1), 70–76.

Böger, B.R., Mori, A.L.B., Viegas, M.C. & Benassi, M.T. (2021). Quality attributes of roasted Arabica coffee oil extracted by pressing: composition, antioxidant activity, sun protection factor and other physical and chemical parameters. *Grasas y Aceites*, **72**, e394.

Burgos-Díaz, C., Opazo-Navarrete, M., Soto-Añual, M. *et al.* (2020). Food-grade Pickering emulsion as a novel astaxanthin encapsulation system for making powder-based products: Evaluation of astaxanthin stability during processing, storage, and its bioaccessibility. *Food Research International*, **134**, 109244.

Carlan, I., Estevinho, B.N. & Rocha, F. (2017). Study of microencapsulation and controlled release of modified chitosan microparticles containing vitamin B12. *Powder Technology*, **318**, 162–169.

Castro-Muñoz, R., González-Valdez, J. & Ahmad, M.Z. (2020). High-performance pervaporation chitosan-based membranes: new insights and perspectives. *Reviews in Chemical Engineering*, 1–16.

Costa, A.L.R., Gomes, A. & Cunha, R.L. (2018). One-step ultrasound producing O/W emulsions stabilized by chitosan particles. *Food Research International*, **107**, 717–725.

Díaz-Montes, E. & Castro-Muñoz, R. (2021). Trends in Chitosan as a primary biopolymer for functional films and coatings manufacture for food and natural products. *Polymers*, **13**, 767.

Eriksen, J.N., Luu, A.Y., Dragsted, L.O. *et al.* (2017). Adaption of an in vitro digestion method to screen carotenoid liberation and in vitro accessibility from differently processed spinach preparations. *Food Chemistry*, **224**, 407–413.

Fang, S., Zhao, X., Liu, Y. *et al.* (2019). Fabricating multilayer emulsions by using OSA starch and chitosan suitable for spray drying: application in the encapsulation of β-carotene. *Food Hydrocolloids*, **93**, 102–110.

Francisco, C.R.L., de Oliveira Júnior, F.D., Marin, G., Alvim, I.D. & Hubinger, M.D. (2020). Plant proteins at low concentrations as natural emulsifiers for an effective orange essential oil microencapsulation by spray drying. *Colloids and Surfaces A*, **607**, 125470.

Freiberger, E.B., Kaufmann, K.C., Bona, E. *et al.* (2015). Encapsulation of roasted coffee oil in biocompatible nanoparticles. *LWT - Food Science and Technology*, **64**, 381–389.

Freiberger *et al.* shared the potential application of two different biopolymers to produce nanoparticles capable of encapsulating bioactive compounds contained in roasted coffee oil. They reported the profile of volatile compounds present in the lipid phase and how these compounds were protected after the encapsulation process by predefined emulsion templates.

Gómez-Mascaraque, L.G., Perez-Masia, R., González-Barrio, R., Periago, M.J. & López-Rubio, A. (2017). Potential of microencapsulation through emulsion-electrospraying to improve the bioaccessibility of β-carotene. *Food Hydrocolloids*, **73**, 1–12.

Ho, K.W., Ooi, C.W., Mwangi, W.W. *et al.* (2016). Comparison of self-aggregated chitosan particles prepared with and without ultrasonication pretreatment as Pickering emulsifier. *Food Hydrocolloids*, **52**, 827–837.

Hurtado-Benavides, A., Dorado A., D. & Sánchez-Camargo, A.D.P. (2016). Study of the fatty acid profile and the aroma composition of oil obtained from roasted Colombian coffee beans by supercritical fluid extraction. *The Journal of Supercritical Fluids*, **113**, 44–52.

Jiang, Y., Zhu, Y., Li, D. *et al.* (2020). Gliadin/amidated pectin core–shell nanoparticles for stabilisation of Pickering emulsion. *International Journal of Food Science and Technology*, **55**, 3278–3288.

José Jara-Palacios, M., Gonçalves, S., Hernanz, D., Heredia, F.J. & Romano, A. (2018). Effects of in vitro gastrointestinal digestion on phenolic compounds and antioxidant activity of different white winemaking byproducts extracts. *Food Research International*, **109**, 433–439.

Karthik, P. & Anandharamakrishnan, C. (2013). Microencapsulation of docosahexaenoic acid by spray-freeze-drying method and comparison of its stability with spray-drying and freeze-drying methods. *Food and Bioprocess Technology*, **6**, 2780–2790.

Li, W., Huang, D., Jiang, Y. *et al.* (2021). Preparation of pickering emulsion stabilised by Zein/Grape seed proanthocyanidins binary composite. *International Journal of Food Science and Technology*, **56**, 3763–3772.

Li, Y., Wu, C., Wu, T. *et al.* (2018). Preparation and characterization of citrus essential oils loaded in chitosan microcapsules by using different emulsifiers. *Journal of Food Engineering*, **217**, 108–114.

Minekus, M., Alminger, M., Alvito, P. *et al.* (2014). A standardised static in vitro digestion method suitable for food – an international consensus. *Food & Function*, **5**, 1113–1124.

Mwangi, W.W., Ho, K.-W., Ooi, C.-W. *et al.* (2016). Facile method for forming ionically cross-linked chitosan microcapsules from Pickering emulsion templates. *Food Hydrocolloids*, **55**, 26–33.

Oliveira, A.L., Cruz, P.M., Eberlin, M.N. & Cabral, F.A. (2005). Brazilian roasted coffee oil obtained by mechanical expelling: compositional analysis by CG-MS. *Ciência e Tecnologia de Alimentos, Campinas*, **25**(4), 677–682.

Pérez-Guzmán, C.J. & Castro-Muñoz, R. (2020). A review of zein as a potential biopolymer for tissue engineering and nanotechnological applications. *Processos*, **8**, 1376.

R.G. Kumar, L., Chatterjee, N.S., Tejpal, C.S. *et al.* (2017). Evaluation of chitosan as a wall material for microencapsulation of squalene by spray drying: characterization and oxidative stability studies. *International Journal of Biological Macromolecules*, **104**, 1986–1995.

Rezvankhah, A., Emam-Djomeh, Z. & Askari, G. (2020). Encapsulation and delivery of bioactive compounds using spray and freeze-drying techniques: a review. *Drying Technology*, **38**, 235–258.

The authors reviewed the literature to report important issues regarding the processes of spray-drying and freeze-drying to encapsulate and release compounds of interest. They highlighted the use of spray-drying as a technique to encapsulate thermo-resistant bioactive compounds. On the other hand, freeze-drying is related to more sensitive core materials.

Ribeiro, E.F., Barros-Alexandrino, T.T., Assis, O.B.G. *et al.* (2020a). Chitosan and crosslinked chitosan nanoparticles: synthesis, characterization and their role as Pickering emulsifiers. *Carbohydrate Polymers*, **250**, 116878.

Ribeiro, E.F., Borreani, J., Moraga, G. *et al.* (2020b). Digestibility and bioaccessibility of pickering emulsions of roasted coffee oil stabilized by chitosan and chitosan-sodium tripolyphosphate nanoparticles. *Food Biophysics*, **15**, 196–205.

This article is a valuable source for previous characterization of the chitosan nanoparticles and the emulsions stabilized with such nanoparticles used for producing the microcapsules studied in the present work.

Schulz, M., Biluca, F.C., Gonzaga, L.V. *et al.* (2017). Bioaccessibility of bioactive compounds and antioxidant potential of juçara fruits (Euterpe edulis Martius) subjected to in vitro gastrointestinal digestion. *Food Chemistry*, **228**, 447–454.

Shu, X.Z. & Zhu, K.J. (2000). A novel approach to prepare tripolyphosphate/chitosan complex beads for controlled release drug delivery. *International Journal of Pharmaceutics*, **201**(1), 51–58.

Silva, V.M., Vieira, G.S. & Hubinger, M.D. (2014). Influence of different combinations of wall materials and homogenisation pressure on the microencapsulation of green coffee oil by spray drying. *Food Research International*, **61**, 132–143.

Singleton, V.L. & Rossi, J.A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture*, **16**, 144–158.

Šturm, L., Osojnik Črnivec, I.G., Istenič, K. *et al.* (2019). Encapsulation of non-dewaxed propolis by freeze-drying and spray-drying using gum Arabic, maltodextrin and inulin as coating materials. *Food and Bioproducts Processing*, **116**, 196–211.

Taboada, M.L., Heiden-Hecht, T., Brückner-Gühmann, M. *et al.* (2021). Spray drying of emulsions: Influence of the emulsifier system on changes in oil droplet size during the drying step. *Journal of Food Processing and Preservation*, **45**, e15753.

Taboada *et al.* recently reported the emulsification template effect on the physico-chemical properties of the microcapsules produced by spray-drying. Authors shared the influence of the addition of lecithin to WPI-stabilized emulsions on the changes in oil droplets during the drying step.

Yan, H., Chen, X., Song, H. *et al.* (2017). Synthesis of bacterial cellulose and bacterial cellulose nanocrystals for their applications in the stabilization of olive oil pickering emulsion. *Food Hydrocolloids*, **72**, 127–135.

Zanin, R.C., Smrke, S., Kurozawa, L.E. *et al.* (2021). Modulation of aroma release of instant coffees through microparticles of roasted coffee oil. *Food Chemistry*, **341**, 128193.