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Microencapsulation by spray drying of emulsified green coffee oil with two-layered membranes

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ABSTRACT

The aim of this work was to produce microparticles of green coffee oil by spray drying using emulsions stabilised by lecithin and chitosan through electrostatic layer-by-layer deposition technique. The emulsions were prepared using only corn syrup and the modified starch Hi-Cap 100 and the combination of the corn syrup with the Hi-Cap 100 or modified starch Snow-Flake at a 50:50 ratio. The feed emulsions were characterised for stability, droplet size, ζ-potential and optical microscopy. The microparticles obtained after the drying process were characterised regarding encapsulation efficiency, moisture content, water activity, particle size distribution, microstructure, in vitro sun protection factor and lipid oxidation by Rancimat. The emulsions stabilised by lecithin–chitosan were highly stable, with droplet size ranged from 1.35 to 3.70 μm and ζ-potential varied from −2.24 to +40.40 mV. All microparticles presented high encapsulation efficiency values, above 86%. The microparticles produced with the modified starches showed spherical shape without cracks or holes and those produced with only corn syrup showed some holes and cracks that caused lower oxidative stability. Microparticles produced with Hi-Cap 100 and corn syrup/Hi-Cap 100 stabilised by lecithin–chitosan exhibited the highest oxidative stability among the microparticles. The sun protection factor of microparticles ranged from 1.52 to 2.45, close to the pure green coffee oil.

1. Introduction

Green coffee oil has been used by the cosmetics industry due to its emollient properties, attributable to the amount of essential fatty acids and because of its antioxidant properties. Additionally, there is evidence that this oil is able to absorb UVB radiation, which causes extensive damage to the human skin (Grollier & Plessis, 1988; Turatti, 2011; Wagemaker, Carvalho, Maia, Baggio, & Guerreiro-Filho, 2011).

Green coffee oil is composed predominantly of triglycerides and significant amounts of unsaponifiable matter, the diterpenes cafestol and kahweol (Speer & Kolling-Speer, 2006). The fatty acids of this oil are predominantly: palmitic acid (30.2% and 31.3%), stearic acid (8% and 5.9%), oleic acid (10.6% and 12.5%) and linoleic acid (46.3% and 44%), extracted from the species *Coffea arabica* and *Coffea canephora*, respectively. The oil from *C. arabica* is more appropriate for applications in cosmetics, offering the highest sun protection factor (SPF) (Wagemaker et al., 2011).

Due to its emollient properties and absorption capacity of solar UVB radiation, the green coffee oil can be applied in cosmetic products. This oil can be used in emulsions in liquid formulations as well as microencapsulated. When this oil is encapsulated, it will not be in direct contact with the human skin. This reduces allergenic effect of cinnamic acid, besides allowing a gradual release. Therefore, the microencapsulation by spray drying of this oil is an alternative to transform it in powder, maintaining its active compounds, besides facilitating the handling and application in others products.

Microencapsulation is a physical method that allows droplets or particles to be trapped in a film formed by the wall material, so establishing a physical barrier between the active material and other components of the product (Gharsallaoui, Roudaut, Chambin, Voilley, & Saurel, 2007). One important step in oil encapsulation by spray drying is the emulsion preparation containing the wall and the core materials, followed by the atomization of this emulsion in a drying chamber with hot air circulation.

Emulsions formed by multilayer may have a potential application for the food or cosmetics industry because this process affords advantages such as protection of the emulsion droplets against lipid oxidation or aggregation, control or the release of active ingredients, besides improving the stability against environmental stresses due to the increment of thickness interface. The electrostatic layer-by-layer deposition consists on the adsorption of an ionic emulsifier in the surface of the lipid droplets during homogenisation and an oppositely charged polyelectrolyte is added to the system, producing a second layer (Guzey & McClements, 2006; McClements, 2004).

Several investigations have been carried out to produce multilayered emulsions using lecithin/chitosan followed by spray drying (Klinkesorn, Sophanodora, Chinachoti, Decker, & McClements, 2006; Klinkesorn,

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Sophanodora, Chinachoti, McClements, & Decker, 2005; Serfert et al., 2011; Shaw, McClements, & Decker, 2007). Furthermore, there are studies conducted with only emulsions prepared using the electrostatic layer-by-layer deposition technique, such as lecithin/chitosan (Klinkesorn, Sophanodora, Chinachoti, Decker, & McClements, 2005), lecithin/chitosan/pectin (Ogawa, Decker, & McClements, 2004), β-lactoglobulin/pectin (Guzey & McClements, 2007), sodium dodecyl sulfate/chitosan/pectin (Thanasukarn, Pongsawatmanit, & McClements, 2006) and sodium caseinate/k-carrageenan (Perrechil & Cunha, 2013).

Thus, the aim of this work was to investigate the process of microencapsulation by spray drying of green coffee oil using emulsions stabilised by lecithin–chitosan and lecithin only, in order to improve the oxidative stability and maintain the sun protection factor of the oil, for possible application in cosmetics. Some emulsion properties as stability, size distribution and droplet mean diameter, ζ-potential and optical microscopy were determined. The microparticles obtained by spray drying were characterised for moisture content, water activity, particle size distribution, microstructure, encapsulation efficiency and in vitro sun protection factor. Finally, they were evaluated with respect to oxidative stability by the Rancimat method.

2. Material and methods

2.1. Material

Green coffee oil (*C. arabica*) cosmetic grade was purchased from Distriol Comércio de Insumos (São Paulo, Brazil) and was used as active material with the following fatty acid composition: 44.31% 18:2; 31.46% 16:0; 10.19% 18:1; 7.82% 18:0; 1.51% 18:3; 3.00% 20:0; 0.78% 22:0; 0.32% 20:1; 0.30% 24:0; 0.13% 17:0; 0.09% 14:0; 0.05% 16:1 and 0.04% 15:0. Wall materials used were corn syrup MOR-REX® 1930 (26–30 DE) (ζ-potential in pH 3.0 is −2.80 ± 0.20) and two chemically octenyl succinic anhydride (OSA) modified starches derived from waxy maize: Hi-Cap 100® (Moisture content 5.51 ± 0.08%; protein 0.23 ± 0.02%; ashes 0.99 ± 0.04% and lipids 0.576 ± 0.003%; ζ-potential in pH 3.0 is −2.80 ± 0.20) and Snow-Flake® E-6131 (moisture content 0.96 ± 0.20%; protein 0.24 ± 0.02%; ashes 0.21 ± 0.02% and lipids 0.396 ± 0.001%; ζ-potential in pH 3.0 is −2.60 ± 0.30) supplied by Ingredion Brasil Ingredientes Industriais Ltd. (Mogi Guaçu, Brazil). Soy lecithin used as emulsifier was supplied by Sina Indústria de Óleos Vegetais Ltd. (São Paulo, Brazil) and powdered chitosan (low molecular weight, deacetylation, 75%–85%) was supplied by Sigma-Aldrich Brazil Ltda. (São Paulo, Brazil). Deionised water was used for the dispersion preparation.

2.2. Emulsion formation

The emulsions stabilised by lecithin–chitosan or lecithin were prepared according to the methodology of Klinkesorn, Sophanodora, Chinachoti, McClements, et al. (2005) with modifications. A stock buffer solution was prepared by dispersing 2 mM sodium acetate and 98 mM acetic acid in water and then adjusted to a pH of 3.0.

Firstly, the wall material corn syrup, Hi-Cap 100, Hi-Cap 100/corn syrup (50:50) and Snow-Flake/corn syrup (50:50) at 64.8% w/w were completely dispersed in pH 3.0 buffer. Lecithin (3.72% w/w) was dispersed into stock buffer solution and homogenised in homogeniser rotor-stator (ULTRA-TURRAX MA-102, Marconi, Piracicaba, Brazil) for about 6 min at 16,000 rpm. Chitosan powder 1.2% (w/w) was dispersed in buffer under magnetic stirring. The pH of the lecithin and chitosan dispersions were adjusted to 3.0 using HCl and stirred overnight to ensure complete dispersion.

Fig. 1 presents the steps of emulsion preparation. Green coffee oil-in-water emulsion (19.2% w/w of oil and 3.0% w/w lecithin in 100 g of emulsion) was prepared by blending 19.2% w/w green coffee oil with 80.80% w/w aqueous lecithin dispersion (3.72% w/w) using a homogeniser ULTRA-TURRAX (MA-102, Marconi, Piracicaba, Brazil) for 3 min operating at 16,000 rpm, followed by three passes through a high-pressure valve homogeniser (PANDA 2K — NS1001L, Niro Soave, Parma, Italy), at 350 bar, with some modifications of Klinkesorn, Sophanodora, Chinachoti, McClements, et al. (2005). The primary emulsion (oil + lecithin) was quite unstable. Coalescence of oil droplets happened almost immediately after the homogenisation process in the first pass and three passes were required to reduce the droplet size and increase stability.

This primary emulsion (66.67% w/w) was diluted with 33.33% (w/w) of aqueous chitosan dispersion (1.2% w/w) to form a secondary emulsion (12.8% green coffee oil, 2% lecithin and 0.4% chitosan) and homogenised using a rotor-stator (ULTRA-TURRAX MA-102, Marconi, Piracicaba, Brazil) for about 3 min at 16,000 rpm, followed by one pass through a high-pressure valve homogeniser (PANDA 2K — NS1001L, Niro Soave, Parma, Italy), at 280 bar, Klinkesorn, Sophanodora, Chinachoti, McClements, et al. (2005) with some modifications. After adding the chitosan dispersion, the system became stable and therefore needed only one pass in the high-pressure homogeniser.

Finally, this emulsion was diluted with 50% (w/w) of each dispersion of corn syrup, Hi-Cap 100, Hi-Cap 100/corn syrup (50:50) and Snow-Flake/corn syrup (50:50) and homogenised in a rotor-stator (ULTRA-TURRAX MA-102, Marconi, Piracicaba, Brazil) for 4 min at 16,000 rpm. The final emulsion was composed of 1% (w/w) of lecithin, 0.2% (w/w) of chitosan, 6.4% (w/w) of green coffee oil and 32.4% (w/w) of wall material.

Following the same procedure, the emulsions stabilised by lecithin without the addition of chitosan dispersion were produced. The green coffee oil-in-water emulsion (12.80% w/w, 2% w/w lecithin) was made by blending 12.80% w/w of green coffee oil with 87.2% w/w of aqueous emulsifier dispersion (2% w/w) using a homogeniser rotor-stator (ULTRA-TURRAX MA-102, Marconi, Piracicaba, Brazil) for 3 min at 16,000 rpm, followed by three passes at 350 bar through a high pressure homogeniser (Panda 2K — NS1001L, Niro Soave, Parma, Italy). Next the final emulsion was prepared by blending 50% (w/w) of the initial emulsion with the dispersions of corn syrup, Hi-Cap 100, Hi-Cap

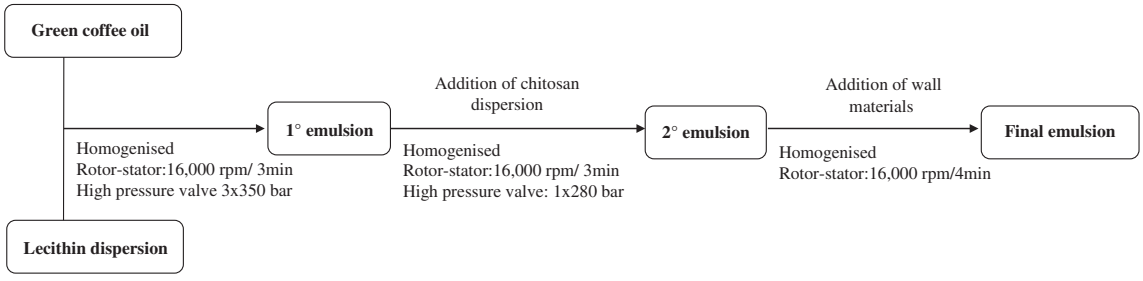

Fig. 1. Flow diagram of green coffee oil-in-water emulsion preparation.

100/corn syrup (50:50) and Snow-Flake/corn syrup (50:50) at 65.2% using a homogeniser rotor-stator (ULTRA-TURRAX MA-102, Marconi, Piracicaba, Brazil), for 4 min at 16,000 rpm.

2.3. Emulsion characterisation

2.3.1. Emulsion stability

Immediately after the emulsion preparation, 25 ml aliquots of each emulsion were poured into a cylindrical graduated glass tube (internal diameter = 1.8 cm, height = 16.5 cm), sealed and stored at 25 °C for a period of one day. The emulsion stability was analysed by the volume of the upper phase measured after 24 h. The phase separation was calculated according to Eq. (1)

$$CI(\%) = \left(\frac{V}{V_0}\right) \times 100 \quad (1)$$

where CI is the creaming index, V_0 represents the initial emulsion volume and V is the upper phase volume.

2.3.2. Emulsion droplet size

The droplet size distribution was characterised using a laser light diffraction instrument, Mastersizer 2000 (Malvern Instrument Ltd., UK). The emulsion droplet size was expressed as d_{32} ($d_{32} = \Sigma n_i d_i^3 / \Sigma n_i d_i^2$), where n_i is the number of the droplets of diameter d_i.

2.3.3. ζ-Potential measurements

In order to determine the electrical charge on the surface of the oil droplets, freshly prepared emulsions were diluted to a droplet concentration of approximately 0.0025% v/v using pH 3.0 buffer solution and placed into the measurement chamber of a microelectrophoresis instrument (Nano ZS Zetasizer, Malvern Instruments Ltd., Worcestershire UK). The measurements were performed in quintuplicate.

2.3.4. Optical microscopy

The emulsions optical microscopy was performed immediately after their preparation. The samples were poured onto microscopes slides, covered with glass cover slips and observed using a Carl Zeiss Model Axio Scope.A1 optical microscope (Zeiss, Germany) with the ×100 objective lenses.

2.4. Spray-drying process

Spray drying was performed in a laboratory scale spray dryer Labplant UK Ltd., model SD 06 (Hunmanby, UK) with a nozzle atomization system with 0.5-mm-diameter nozzle and spray chamber of 0.5 × 0.215 m. The emulsion was fed into the primary chamber through a peristaltic pump, at 25 °C under magnetic stirring. The drying air flow rate was 300 m^3/h, air pressure was 4 bar, the compressed air flow rate was 1.7 m^3/h and the feed flow rate was 11.6 mL/min. Experimental tests were carried out with an inlet air drying temperature of 170 °C (Frascareli, Silva, Tonon, & Hubinger, 2012) and outlet air temperature was 90 ± 1 °C. The production of microparticles by spray drying was performed in duplicate.

2.5. Encapsulation efficiency (EE)

Encapsulation efficiency (EE) was determined according to the method described by Bae and Lee (2008). Fifteen millilitres of hexane were added to 1.5 g of powder in a glass jar with a lid. This was shaken by hand for 2 min at room temperature for the oil extraction. The solvent mixture was filtered through a Whatman filter paper grade 1 and the powder collected on the filter was rinsed three times with 20 mL of hexane. Then, the solvent was left to evaporate at room temperature and after at 60 °C, until constant weight. The non-encapsulated oil (surface oil) was determined by mass difference between the initial clean flask and that containing the oil residue extracted. Total oil was assumed to be equal to the final emulsion oil, because green coffee oil is not volatile. Encapsulation efficiency (EE) was calculated from Eq. (2)

$$EE = \frac{\left(Oil_{Total} - Oil_{Surface}\right)}{Oil_{Total}} \times 100 \quad (2)$$

where Oil_{Total} is the total oil content based on the dry matter of the final emulsion and $Oil_{Surface}$ is the surface oil content.

2.6. Powder characterisation

2.6.1. Moisture content and water activity

Moisture content was determined gravimetrically by drying in forced-circulation oven at 105 °C until constant weight (AOAC, 1997). The water activity of samples was measured by AquaLab Water Activity Meter (series 3TE, Decagon, Pullman, USA), at 25 °C.

2.6.2. Particle size distribution

The particle mean diameter was measured using a laser light diffraction instrument, Mastersizer 2000 (Malvern Instruments Ltd, Malvern, UK). A small sample was suspended in ethanol (99.5%) and the particle size distribution was monitored during each measurement until 5 successive readings have become constant. The particle size was expressed as d_{43} ($d_{43} = \Sigma n_i d_i^4 / \Sigma n_i d_i^3$) the volume weighted mean diameter.

2.6.3. Microstructure

Microparticles were observed in a Scanning Electron Detector microscope with Energy Dispersive X-ray, LEO 440i — 6070 (LEICA Electron Microscopy Ltd., Cambridge, England), operating at 15 kV and electron beam current of 150 pA. The samples were fixed directly on door-metallic specimens (stubs) of 12 mm diameter and 10 mm height and then subjected to metallization (sputtering) with a thin layer of gold in a Sputter Coater SC7620, VG Microtech (Uckfield, England) at a coverage rate of 0.17 Å/mA V s for 180 s, with a current of 3 mA, 1 V and thickness of 92 Å. After metallization, the samples were observed with magnification of 7000×. Image acquisition was performed by the LEO software, version 3.01.

2.6.4. Sun protection factor

In vitro sun protection factor (SPF) of microparticles was determined by the spectrophotometric method developed by Mansur, Breder, Mansur, and Azulay (1986), with some modifications. The pure green coffee oil was dissolved in ethanol at concentration of 0.2 μl/mL and 0.12 g of the microparticles was dissolved in 20 ml of deionised water and then dispersed in ethanol at 100 ml to obtain the same oil concentration. The samples were centrifuged for 30 min at 10,000 rpm for extraction of the oil and filtered in quantitative filter paper J. Prolab (JP42 — 8 μm diameter) to remove the wall material. The filtered material was submitted to reading in spectrophotometer UV/VIS model SP-220 (Biospectro/Merse, São Paulo, Brazil) ranging from 290 to 320 nm with intervals of 5 nm. SPF was calculated using Eq. (3)

$$SPF = CF \cdot \sum_{290}^{320} EE(\lambda) \cdot I(\lambda) \cdot Abs(\lambda) \quad (3)$$

where CF = correction factor (10), $EE(\lambda)$ = erythmogenic effect of radiation with wavelength λ, $Abs(\lambda)$ = spectrophotometric absorbance values at wavelength λ. The values of $EE(\lambda) \cdot I(\lambda)$ are constants (Mansur et al., 1986).

2.6.5. Lipid oxidation

The Rancimat method is widely accepted for determining oxidative stability of solids that contain fats or oils. The volatiles oxidation of particles and green coffee oil were evaluated according to the induction

time measured by the conductivity provided by products formed during oxidation. Induction time is defined by the time required to reach the inflection point in the curve, which is calculated by the intersection of the tangent line to the curve projected along the time axis. The induction time was measured by the equipment Biodiesel Rancimat 873 (Metrohm, Herisau, Switzerland) on 2.0 g of the sample (microparticles and green coffee oil) heated at 120 °C under a purified air flow rate of 20 L/h, in duplicate.

2.7. Statiscal analysis

Results were statistically analysed by Analysis of Variance, using the software Statistica® 8.0 (Statsoft Inc., Tulsa, USA). Mean analysis was performed using Tukey's procedure at $p \leq 0.05$.

3. Results and discussion

3.1. Emulsion characterisation

3.1.1. Emulsion stability

The green coffee oil emulsified with lecithin showed faster droplet coalescence and phase separation (CI = 8.1 ± 0.2%) after 24 h of preparation. However, the addition of chitosan into the initial system formed by oil + lecithin provided a greater stability, showing no phase separation after 24 h. Chitosan can act as a good stabiliser in oil–water emulsions, because it can adsorb at the interface oil/water protecting the droplets against coalescence, besides contributing towards mechanical and electrostatic stability of the oil droplets. Chitosan presents heterogeneity of the polyelectrolyte composition, i.e., molecules less deacetylated are more hydrophobic and the others are more hydrophilic (Schultz, Rodríguez, Del Branco, Pistonesi, & Agulló, 1998). Therefore its absence in the initial emulsion (oil + lecithin) may have contributed to a greater instability.

After the addition of the wall material corn syrup, Hi-Cap 100, corn syrup/Hi-Cap 100 (50:50) and corn syrup/Snow-Flake (50:50), all emulsions were considered kinetically stable, showing no phase separation after 24 h. Only the emulsion formed by corn syrup/Hi-Cap 100 (50:50) and stabilised by lecithin showed a suspended material at the surface, which may indicate a separation of lecithin and oil. There was a reduction of the chemical groups with emulsifying capacity in this emulsion due to corn syrup addition and absence of the chitosan. According to Guzey and McClements (2006) the emulsions stabilised by lecithin–chitosan got a much better stability to droplet aggregation than emulsions stabilised by only lecithin, especially when maltodextrin was incorporated into the system. Polysaccharide addition to oil/water emulsions allows the increment of viscosity of the aqueous phase and prevents creaming of the oil droplets (McClements, 2000).

Corn syrup addition after emulsification process of green coffee oil with lecithin provided phase separation, due to flocculation of oil droplets. Then, it was not possible to obtain an emulsion prepared with corn syrup stabilised by lecithin. Maltodextrin and corn syrup non-adsorbed in the aqueous phase of the emulsion can promote rapid creaming when a critical polymer concentration was exceeded. Therefore, the attractive force increases with the raise of concentration of these biopolymers, until the moment it becomes sufficiently high to overcome the repulsive forces between the oil droplets and promotes flocculation due to the osmotic effect (Klinkesorn, Sophanodora, Chinachoti, & McClements, 2004; McClements, 2000). Additionally, the modified starches Hi-Cap 100 and Snow-Flake exhibit emulsifying capacity due to the presence of lipophilic groups in their structure; conversely corn syrup does not show this property.

3.1.2. Emulsion droplet size

The droplet mean diameter (d_{32}) of the emulsions produced at each stage was determined. Primary emulsion (oil + lecithin) showed higher droplet mean diameter (3.63 ± 0.30 μm) compared to the secondary emulsion (oil + lecithin + chitosan) that presented droplet mean diameter of 1.44 ± 0.02 μm. The largest diameter of the droplets in primary emulsion may be related to the greater instability of this emulsion, that may have caused the rapid coalescence of droplets after the homogenisation process.

Table 1 shows the emulsion droplet mean diameter after the addition of the wall material corn syrup, Hi-Cap 100, corn syrup/Hi-Cap 100 (50:50) and corn syrup/Snow-Flake (50:50). Emulsions formed by corn syrup/Snow-Flake (50:50) exhibited larger droplet mean diameters compared to the other wall materials.

According to Fig. 2, the curves revealed a bimodal droplets size distribution with two peaks representing the predominant size. The emulsions produced with Hi-Cap 100 and stabilised by lecithin–chitosan and by only lecithin presented a large peak around 1 μm and a minor peak, in the range of 10 to 100 μm. Those produced with corn syrup/Snow-Flake (50:50) and stabilised by lecithin–chitosan and by only lecithin showed a large peak around 10 μm and a minor peak around 1 μm. Fig. 3 shows optical microscopy of the emulsions and the presence of larger droplets in the emulsions with corn syrup/Snow-Flake (D and G) and smaller droplets in the emulsions with corn syrup (A), Hi-Cap 100 (B and E) and corn syrup/Hi-Cap 100 (C and F) can be observed.

3.2. ζ-Potential measurements

The ζ-potential lecithin dispersion in buffer pH 3.0 was negative (−19.50 ± 0.40 mV) and the dispersion of chitosan in buffer pH 3.0 was positive (+33.0 ± 0.70 mV). Lecithin is an ionic phospholipid and its dissociation behaviour depends on the pH in which it is dispersed (Fellows, 2009). The phosphate groups on lecithin typically have pKa values around 1.5 and the cationic groups of chitosan have pKa values around 6.2 and 7.0, in acidic system, such as pH 3.0. Then, it is possible to occur protonation of the amino group of chitosan (NH_3^+), presenting thus cationic character and complete dissociation of lecithin, keeping its anionic character because the pH 3.0 is above its pKa value (Chuah, Kuroiwa, Ichikawa, Kobayashi, & Nakajima, 2009).

The ζ-potential of the primary emulsion (green coffee oil + lecithin) was negative (−29.6 ± 0.70 mV), which may be related to the presence of free fatty acids and to adsorption of anionic phospholipid on the droplets too. However, the addition of chitosan dispersion provided that droplet surface acquired positive character, as soon as the ζ-potential of the secondary emulsion (oil + lecithin + chitosan) was positive (+66.70 ± 0.80 mV), suggesting that the chitosan molecules formed a second layer. The choice of the emulsifier will determine the electrical characteristic of the first layer in the emulsion droplets and influence on

Table 1
Characterisation of emulsions prepared with different types of wall materials.

Formulations	d_{32} (μm)		ζ-Potential (mV)	
	Lecithin–chitosan	Lecithin	Lecithin–chitosan	Lecithin
Corn syrup	1.51 ± 0.03^{b}	–	+40.40 ± 0.53^{a}	–
Hi-Cap 100	1.35 ± 0.100^{c,A}	1.15 ± 0.04^{c,B}	−2.24 ± 0.06^{d,B}	−3.64 ± 0.15^{c,A}
Corn syrup/Hi-Cap 100 (50:50)	1.37 ± 0.13^{b,c,A}	1.36 ± 0.01^{b,A}	+13.41 ± 0.23^{c,A}	−3.88 ± 0.15^{c,B}
Corn syrup/Snow-Flake (50:50)	3.70 ± 0.07^{a,A}	3.76 ± 0.06^{a,A}	+24.32 ± 0.62^{b,A}	−16.90 ± 0.30^{a,B}

Different small letters in the some column indicate a significant difference, as well as different capital letters in the same line ($p \leq 0.05$).

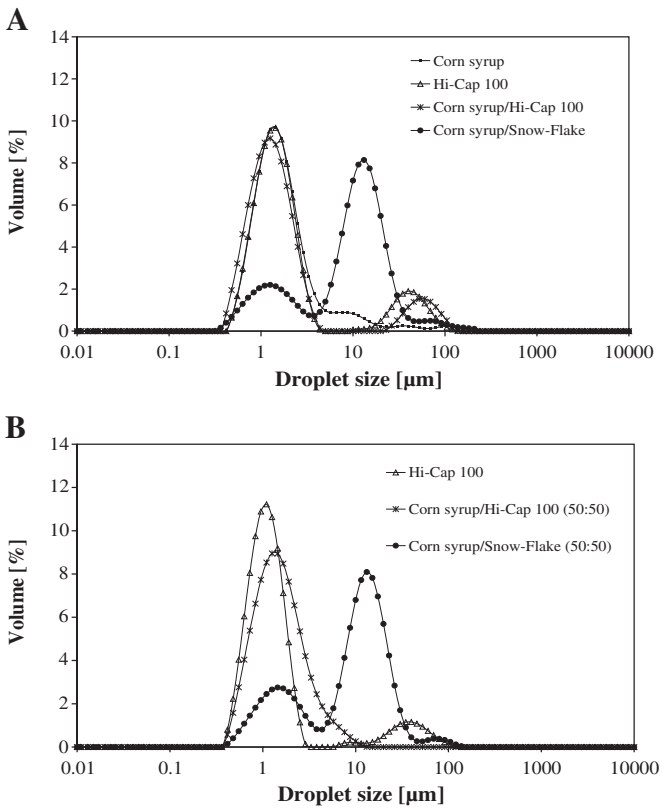

Fig. 2. Droplet size distribution of emulsions stabilised by lecithin–chitosan (A) and lecithin (B) prepared with different wall materials.

the formation of this interface. If the total number of charges is greater than needed to neutralise the opposite charges on the surface it induces charge reversal (Guzey & McClements, 2006).

The stability of emulsions, dispersions or suspensions may be related with the ζ-potential, which is measured by attraction or repulsion between the charges of the particles. According to Chuah et al. (2009) ζ-potential values above +60 mV indicate that the system exhibits excellent stability. The secondary emulsion (oil + lecithin + chitosan) showed great stability and high ζ-potential value in buffer pH 3.0.

Ogawa et al. (2004) observed that the primary emulsion formed by corn oil stabilised by lecithin showed negative charge in the pH range from 3 to 8. The ζ-potential was +38 mV with the addition of chitosan dispersion. These authors verified that as pH values increased, the global balance charge of the emulsion reduced the positive charges and above pH 5, the global balance became negative. Moreover, ζ-potential became slightly positive (+8 mV) with addition of pectin, suggesting that the negative charges of pectin were insufficient to overcome the high positive charge of chitosan–lecithin surface.

The wall material behaviour in pure form or in combination was also analysed in buffer pH 3.0. All wall materials showed rather neutral charges, in the range of −2.60 to −2.80 mV. The modified starches are polysaccharides with emulsifying properties and, when used in the preparation of the emulsions tend to form an anionic interface, however they aren't compounds with high active surface and require high polysaccharide-to-oil ratios in the formation of the emulsion (Guzey & McClements, 2006). According to Klinkesorn, Sophanodora, Chinachoti, Decker, et al. (2005) corn syrup solids are considered hydrophilic non-ionic oligosaccharides and they do not contribute to form an active surface or to charge absorption. In this study, corn syrup showed rather neutral charges in buffer pH 3.0.

The ζ-potential values of the emulsions stabilised by lecithin–chitosan and lecithin combined with the different wall materials are displayed in Table 1. All emulsions stabilised by only lecithin showed surface of droplets with negative charge, meanwhile the ones stabilised with lecithin–chitosan showed droplets surface with positive charge, except for the emulsion produced with Hi-Cap 100. The emulsion stabilised by lecithin–chitosan prepared with Hi-Cap 100 presented negative ζ-potential (−2.24 ± 0.06 mV), probably the total number of negative charges of this wall material was greater than the required to neutralise the positive charge of chitosan. This could have caused phase inversion on the surface of the oil droplets.

3.3. Spray drying

3.3.1. Moisture content and water activity

Table 2 presents the moisture content and water activity of the microparticles. Powder moisture content varied from 0.41 to 2.49% (w.b.). Water activity of the microparticles was less than 0.3. Microparticles produced with corn syrup showed water activity lower than 0.1 because these particles were retained in the cyclone and this region is hotter than the particle collector. The samples prepared with corn syrup and corn syrup/Snow-Flake stabilised by lecithin had the lowest moisture content and also the lowest values of water activity. Microparticles formed by corn syrup/Hi-Cap 100 (50:50) presented highest water activity and moisture content than microparticles composed by corn syrup/Snow-Flake.

Klinkesorn et al. (2006) produced particles of tuna oil by electrostatic layer-by-layer deposition using lecithin and chitosan and observed that the moisture content ranged from 3 to 1% and water activity ranged from 0.25 to 0.1, as air drying temperature increased from 165 to 180 °C, respectively. Similar values were obtained for the moisture content and water activity in this work at 170 °C.

3.3.2. Encapsulation efficiency

Encapsulation efficiency ranged from 86 to 97% and it was significantly influenced by the kind of wall material and the use of chitosan. According to the results of encapsulation efficiency in Table 2, microparticles prepared with Hi-Cap 100 or combined with corn syrup (50:50) presented the best encapsulation efficiency values. This may be related to the effect of emulsifying property of Hi-Cap 100 that exhibits lipophilic groups on its structure. Microparticles formed by the mixture of modified starches Hi-Cap 100 and Snow-Flake with corn syrup presented better encapsulation efficiency when stabilised by lecithin–chitosan, whereas chitosan may have contributed to a greater stability of these emulsions, providing lower surface oil content in the microparticles and higher values of encapsulation efficiency.

Klinkesorn et al. (2006) observed that tuna oil microspheres containing 5% tuna oil, 1% lecithin, 0.2% chitosan and 20% corn syrup solids (DE 36) presented encapsulation efficiencies between 85 and 87% and these values were not affected by air drying temperature, which varied from 165 to 180 °C. Serfert et al. (2011) verified that the fish oil (9%) encapsulated with glucose syrup (35.2%), lecithin (1%) and chitosan (0.2%) showed an encapsulation efficiency of 83.1%. Similar values were found in this work for the microparticles produced with corn syrup stabilised by lecithin–chitosan.

3.4. Particle size distribution

According to Table 2, particle mean diameter varied from 14.51 to 19.50 μm for emulsions stabilised by lecithin–chitosan and 16.40 to 29.19 μm for emulsions stabilised by lecithin. Microparticles produced with Hi-Cap 100, stabilised by lecithin–chitosan presented larger diameter with the addition of corn syrup than microparticles produced with only Hi-Cap 100.

The particles showed a multimodal distribution with distinct peaks, each one representing a predominant size, as presented in Fig. 4. A similar trend was observed in the distribution of the particle size when comparing single layer and bilayer emulsions. Particle size distribution showed the formation of a predominant peak size ranged from

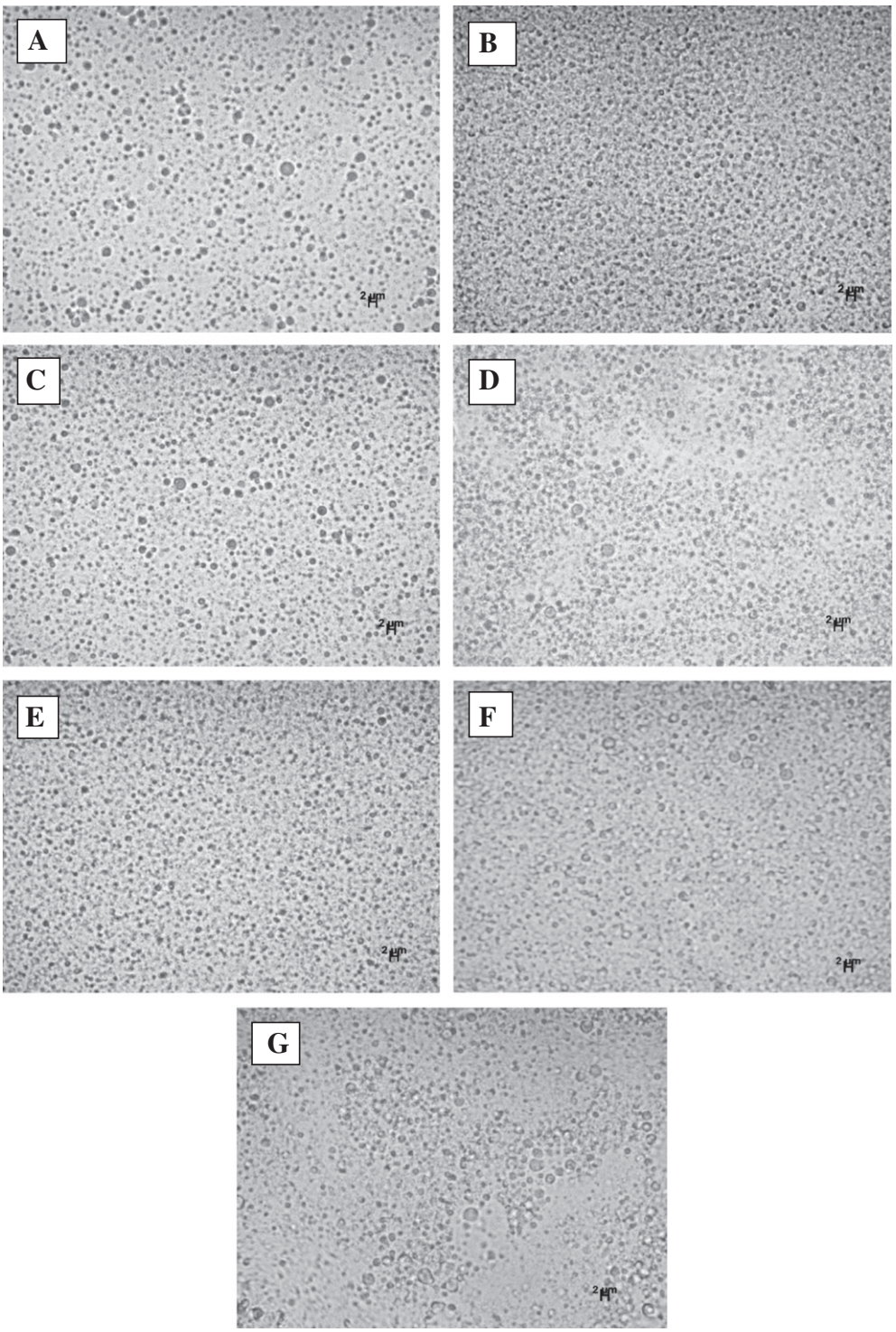

Fig. 3. Microstructure of emulsions immediately after their preparation with the × 100 objective lenses. Emulsions stabilised by lecithin–chitosan prepared with corn syrup (A), Hi-Cap 100 (B), corn syrup/Hi-Cap 100 (50:50) (C) and corn syrup/Snow-Flake (50:50) (D) and emulsions stabilised by lecithin prepared with Hi-Cap 100 (E), corn syrup/Hi-Cap 100 (50:50) (F) and corn syrup/Snow-Flake (50:50) (G).

10 to 20 μm and minor peak particle with a diameter of approximately 1 μm and larger particle sizes ranged from 100 to 200 μm.

3.5. Powder morphology

Fig. 5 reveals the SEM microphotographs (internal and external) of the powders produced using emulsions stabilised by lecithin–chitosan and Fig. 6 reveals the powders produced using emulsions stabilised by only lecithin. The particles exhibited spherical shape and irregular surface and various sizes, which is a typical characteristic of spray dried powders.

The microparticles produced using corn syrup (Fig. 5A) presented some cracks and some holes. This fact can allow higher air permeability in microparticles reducing the protection of the active material. Ahn et al. (2008) encapsulated sunflower oil with milk protein isolate, dextrins and soy lecithin as an emulsifier, verifying that the microspheres with low encapsulation efficiency (70.2%) had on its surface pores and cracks; however, the microspheres with higher

Table 2
Characterisation of microparticles prepared with different wall materials.

Formulations	Water activity		Moisture content (%)		Encapsulation efficiency (%)		d_{43} (μm)	
	Lecithin–chitosan	Lecithin	Lecithin–chitosan	Lecithin	Lecithin–chitosan	Lecithin	Lecithin–chitosan	Lecithin
Corn syrup	0.085 ± 0.006^{d}	–	0.41 ± 0.03^{c}	–	86.8 ± 1.1^{d}	–	19.50 ± 0.74^{a}	–
Hi-Cap 100	0.224 ± 0.007^{bA}	0.228 ± 0.012^{bA}	$2.42 \pm 0.13^{a,A}$	$2.49 \pm 0.09^{a,A}$	$95.7 \pm 0.8^{b,B}$	$96.8 \pm 0.6^{a,A}$	$16.41 \pm 0.95^{c,B}$	$24.40 \pm 0.67^{b,A}$
Corn syrup/Hi-Cap 100 (50:50)	0.263 ± 0.004^{aA}	0.262 ± 0.014^{aA}	$2.41 \pm 0.03^{a,A}$	$2.17 \pm 0.08^{b,B}$	$97.5 \pm 0.7^{a,A}$	$91.4 \pm 0.6^{b,B}$	$17.31 \pm 0.59^{b,B}$	$29.19 \pm 0.84^{a,A}$
Corn syrup/Snow-Flake (50:50)	0.209 ± 0.004^{cA}	0.153 ± 0.005^{cB}	$2.16 \pm 0.05^{b,A}$	$0.42 \pm 0.03^{c,B}$	$91.9 \pm 1.5^{c,A}$	$87.2 \pm 0.4^{c,B}$	$14.51 \pm 0.35^{d,B}$	$16.40 \pm 0.59^{c,A}$

Different small letters in the some column indicate a significant difference, as well as different capital letters in the same line ($p \leq 0.05$).

encapsulation efficiency (96.6%) had a smooth surface and free of pores and cracks.

The microparticles produced with Hi-Cap 100 (Figs. 5C and 6A) presented a spherical shape with no cracks or holes. Soottitantawat et al. (2005) have evaluated the encapsulation of D-limonene by spray drying and verified that particles produced with Hi-Cap 100 had a smoother surface than the powders constituted with gum Arabica and maltodextrin 20 DE.

The combination of corn syrup with the modified starches displayed in Figs. 5E and G and 6C and E gave the formation of particles with varied formats, microparticles with smooth surface and wrinkled too. Figs. 5B, D, F and H and 6B, D and F show the internal surfaces of microparticles. The small pores are droplets of green coffee oil embedded on the shell of the wall matrix.

3.6. Sun protection factor (SPF)

The green coffee oil showed in vitro sun protection factor (SPF) of 2.12 ± 0.06, however Wagemaker et al. (2011) determined for the same kind of coffee oil SPF values ranged from 1.24 to 1.78. Those differences may have been caused by different conditions of harvest of green coffee beans, extraction methods or conditions of analysis.

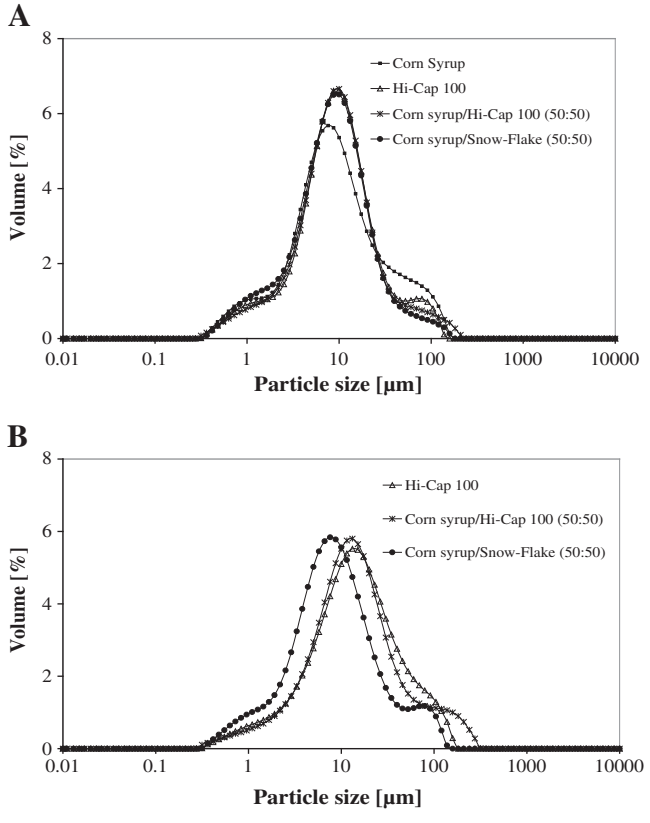

Fig. 4. Particle size distribution of spray dried emulsions stabilised by lecithin–chitosan (A) and lecithin (B) prepared with different wall materials.

The encapsulated oil presented SPF varying from 1.52 to 2.45 and these values are close to that showed by green coffee oil, as can be seen in Table 3. Particles prepared with corn syrup had a SPF higher than the other microparticles. Although a quantitative filter was used to retain wall material in the analysis of the in vitro sun protection factor of green coffee oil, it is possible that corn syrup may have interfered in spectrophotometric measurement and increased the sun protection factor for encapsulated oil with this wall material. The combination of corn syrup with Hi-Cap 100 provided an increase in the SPF, compared with microparticles constituted with only Hi-Cap 100.

The product formulated with the microparticles aims to protect the active compounds of green coffee oil from oxidation. However when they are applied on the skin, these compounds are released either by mechanical action or by pH change and effectively protect the skin. The oil already present in the cosmetic formulation in its free form is subjected to oxidation and may lose its UVB protection effect before being applied.

According to in vitro SPF results (displayed in Table 3), the green coffee oil microparticles maintained its UVB protection. However, other tests accepted and recommended by legislation are necessary to confirm the efficiency of the green coffee oil microparticles and possible application in cosmetic formulations. Spectrophotometric analysis performed in this study only indicates the presence of compounds capable of absorbing ultraviolet radiation in the UVB length.

No restrictions for utilisation of green coffee oil in cosmetics products in either U.S. Food and Drugs Administration (FDA) or Brazilian Health Surveillance Agency (ANVISA) were found. In general, products with unknown risks need to be tested in vitro and in vivo, followed by clinical tests.

3.7. Oxidative stability

The induction time values (h) of the microparticles are displayed in Table 3. This values can be related with oxidative stability, the greater the induction time the higher the oxidative stability. The green coffee oil showed an induction time of 2.36 h. A short induction time was expected for the green coffee oil (control) due to the presence of unsaturated fatty linoleic acid (C18: 2) in an amount of 44.31%. Halbaut, Barbé, Aróztegui, and De La Torre (1997) using the Rancimat method obtained an induction time for the corn oil of approximately 2.26 h, according to these authors this short induction time is characteristic of polyunsaturated oils.

Microparticles produced with Hi-Cap 100 and corn syrup/Hi-Cap 100 showed the best encapsulation efficiency values and, consequently, higher induction times ranging from 13.50 to 26.75 h. Additionally, microparticles prepared with lecithin–chitosan exhibited higher oxidative stability compared to the particles constituted by only lecithin. The presence of chitosan in microparticles produced with Hi-Cap 100 and corn syrup/Hi-Cap 100 provided better barrier to oxidation. Ahn et al. (2008) analysed the oxidation of microparticles of sunflower oil, milk protein isolates and soy lecithin by Rancimat method. These

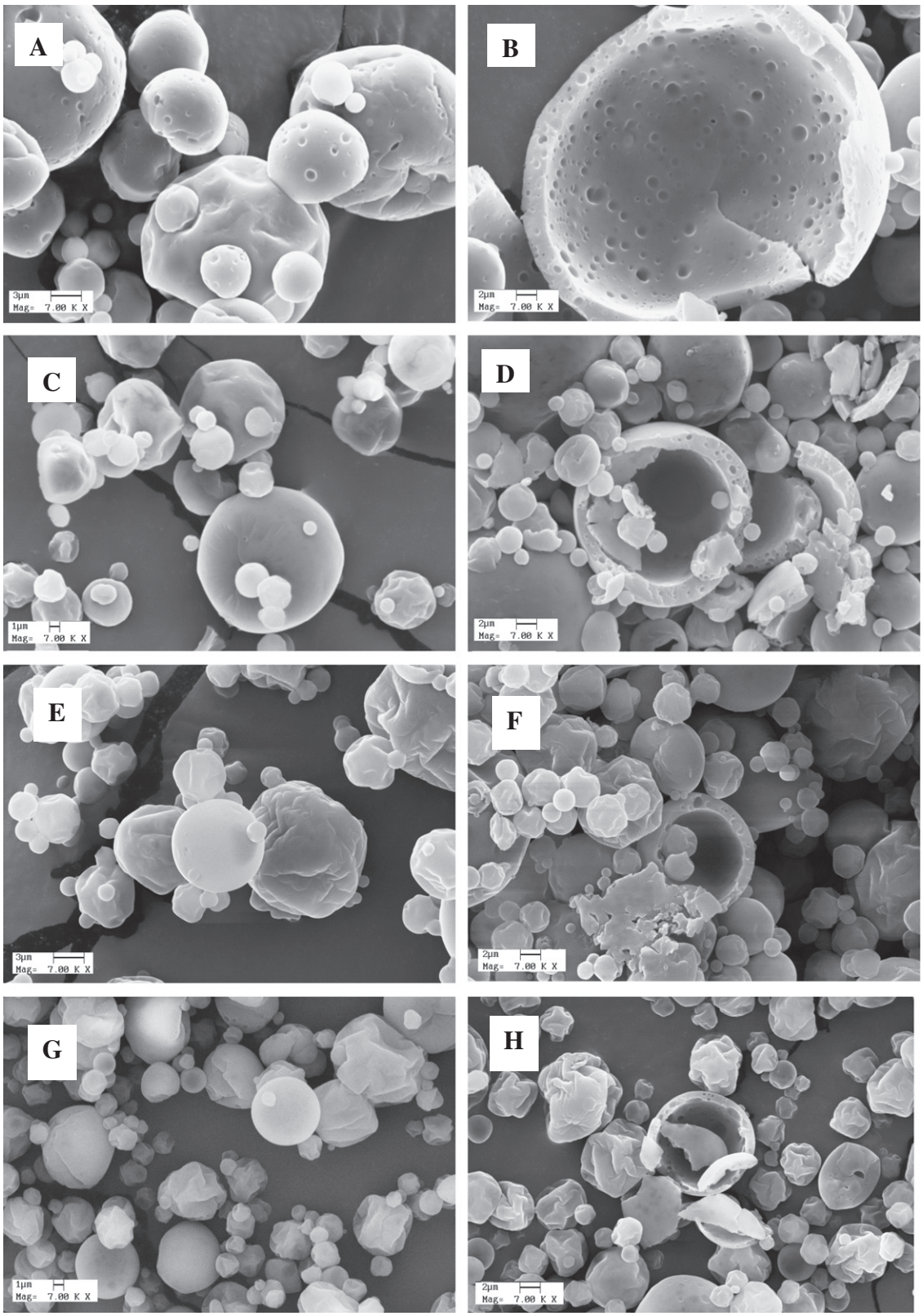

Fig. 5. Representative micrographs showing the outer (left) and internal (right) morphology. Powders formed by spray dried emulsion stabilised by lecithin–chitosan: corn syrup (A, B), Hi-Cap 100 (C, D), corn syrup/Hi-Cap 100 (E, F) and corn syrup/Snow-Flake (G, H) with 7000× of magnification.

authors obtained induction time corresponding to 20.80 h for microparticles produced in optimal conditions, with an encapsulation efficiency of 96.6%, compared to the time of 12.70 h for microparticles with 70.2% of encapsulation efficiency. Hong-Kwong, Chin-Ping, Bakar, and Siou-Pei (2012) studied the oxidative stability of the pitaya seed oil microencapsulated by spray drying using the Rancimat method.

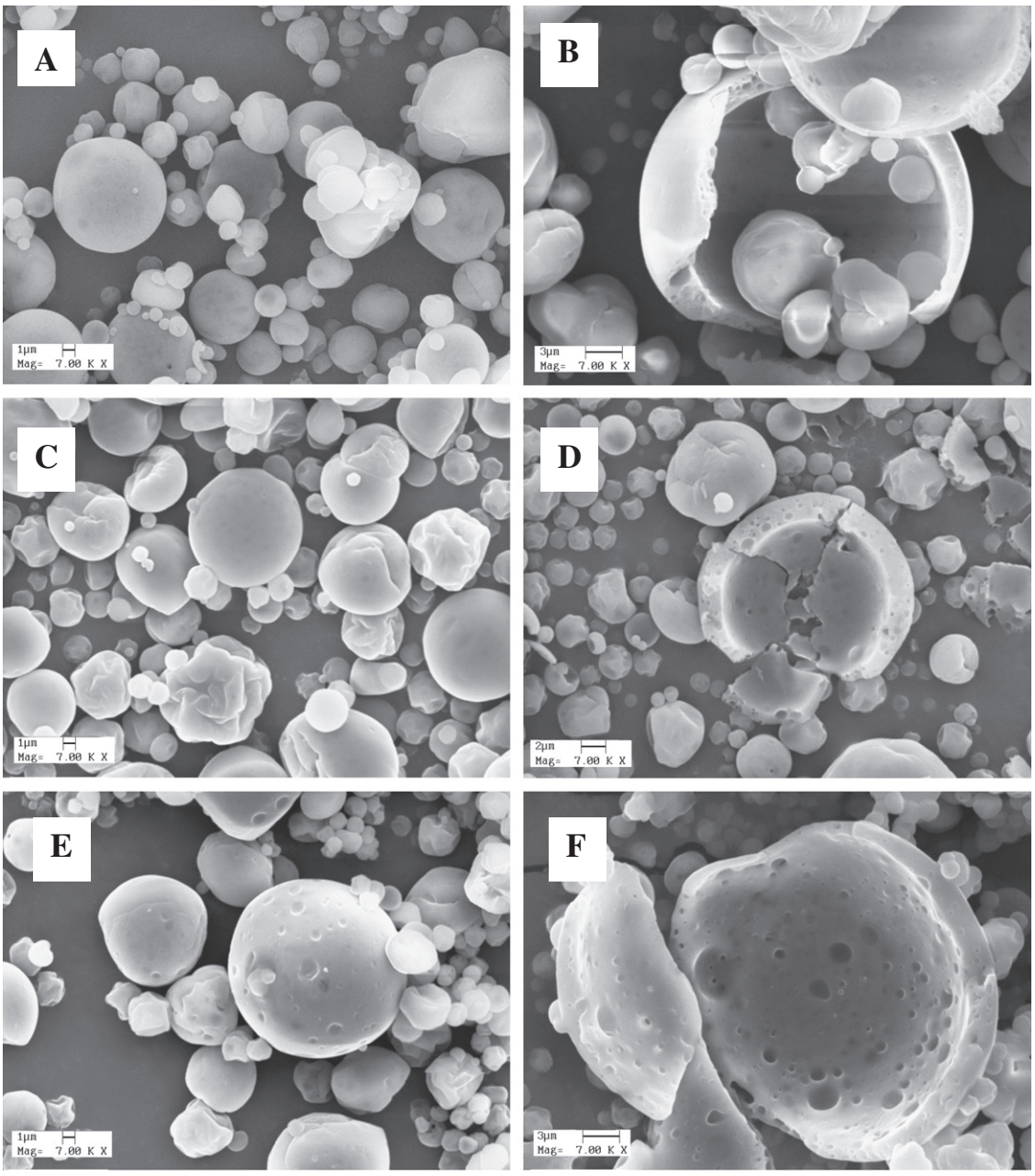

Fig. 6. Representative micrographs showing the outer (left) and internal (right) morphology. Powders formed by spray dried emulsion stabilised by lecithin: Hi-Cap 100 (A, B), corn syrup/Hi-Cap 100 (C, D) and corn syrup/Snow-Flake (E, F) with 7000× of magnification.

These authors observed that the induction time varied from 5.20 to 38 h and this variation was influenced by the different wall materials used.

The lipid oxidation is difficult to occur at water activity values near 0.3, however, water activity values below 0.2 and above 0.5 accelerate the oxidation of lipids (Labuza, Mcnally, Gallagher, Hawkes, & Hurtado,

Table 3
Induction time and SPF for pure green coffee oil and microparticles prepared with different wall materials.

Control	Induction time (h)		SPF	
Green coffee oil	2.36 ± 0.08		2.12 ± 0.06	
Microparticles	Lecithin–chitosan	Lecithin	Lecithin–chitosan	Lecithin
Corn syrup	6.90 ± 0.14^{c}	–	2.45 ± 0.15^{a}	–
Hi-Cap 100	26.75 ± 1.06^{a,A}	15.00 ± 2.82^{a,B}	1.52 ± 0.10^{c,B}	1.92 ± 0.07^{a,A}
Corn syrup/Hi-Cap 100 (50:50)	17.50 ± 0.70^{b,A}	13.50 ± 0.70^{a,B}	1.92 ± 0.06^{b,A}	1.98 ± 0.01^{a,A}
Corn syrup/Snow-Flake (50:50)	6.20 ± 0.14^{c,B}	9.50 ± 0.70^{a,A}	1.57 ± 0.09^{c,A}	1.63 ± 0.05^{b,A}

Different small letters in the some column indicate a significant difference, as well as different capital letters in the same line ($p \leq 0.05$).

1972). Microparticles with corn syrup and corn syrup/Snow-Flake (50:50) showed water activity lower or equal to 0.2, which might have contributed to lower oxidative stability as presented by the induction time of these microparticles (Table 3).

4. Conclusions

The use of the modified starch Hi-Cap 100 and in combination with corn syrup incurred to be a good alternative as wall materials for oil microencapsulation using emulsions stabilised by lecithin and chitosan. The encapsulation efficiency of the microparticles increased with the presence of the modified starches in emulsions. Microparticles produced with Hi-Cap 100 and Hi-Cap 100/corn syrup (50:50) revealed higher values of encapsulation efficiency and they were less susceptible to oxidation. The combination of Snow-Flake with corn syrup and only corn syrup produced microparticles more susceptible to oxidation. The electrostatic layer-by-layer deposition technique can be used for the microencapsulation of green coffee oil, maintaining the sun protection factor of this oil. This technique can be applied for oil microencapsulation to improve oxidative stability.

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