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Gallic acid microparticles produced by spray chilling technique: Production and characterization

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ABSTRACT

In this work, we evaluated the implementation of spray chilling technique to produce solid lipid microparticles (SLMs) containing gallic acid as a model phenolic compound, using blends of soybean oil (SO) with fully hydrogenated soybean oil (FHSO) as wall materials. Gallic acid aqueous solution was dispersed through lipid blends by the preparation of water-in-oil (W/O) emulsions stabilized by the emulsifier PGPR (polyglycerol polyricinoleate). The conditions of emulsion preparation (concentration of emulsifier, speed and time of stirring) were established by evaluating their kinetic stability. The FHSO/SO proportion and the W/O ratio have been varied for the different formulations. Lipid blends and emulsions flow curves showed Newtonian behavior at 79 °C (preparation temperature). Microparticles with higher FHSO concentrations presented increased encapsulation efficiencies (from 54% to 101%) due to lower gallic acid superficial concentrations. Mean diameters ranged from about 24 μm to 36 μm and were slightly affected by differences in formulations. Scanning electron microscopy revealed microparticles with spherical shape and smooth surface covered with fat crystals. Hence, the use of spray chilling technique may be a good option for the production of SLMs loaded with phenolics.

1. Introduction

Phenolic compounds comprise a wide range of compounds with different properties. Their chemical structure basically includes a hydroxylated aromatic ring. In terms of functionality, the importance of these compounds in plants is mainly due to color and flavor (Haard & Chism, 1996). Besides functional properties, many health benefits to humans, such as coronary heart disease prevention, retina protection, modulation of the immune system, and tumor development inhibition, have been associated to these phytochemicals (Munin & Edwards-Lévy, 2011).

Both for technological and nutritional abilities, the use of phenolic compounds as food ingredients is very interesting. Therefore, it is important to ensure that these compounds are actually present in the product since its production until consumption, fully protected from external degradation factors. Microencapsulation can then be used as a potential alternative to protect phenolic compounds.

Spray chilling is a microencapsulation technique similar to spray drying, in which cooled air is used for particles solidification instead of heated air to solvent evaporation (Desai & Park, 2005). Carriers used in this technique are usually lipids such as fatty acids, oils, triacylglycerols, waxes or blends of these materials. Generally, the active material is dispersed through the molten carrier forming emulsions or suspensions, and then this mixture is atomized into a cooled chamber, in which the droplets are solidified. Since lipids can present a broad range of melting temperature, a suitable carrier may be chosen according to the temperature stability of the active material, making possible the application of this technique for encapsulating thermolabile materials. Spray chilling process does not require the use of organic solvents. Besides that, it presents a relatively low cost compared to other techniques and is considered ease to scale up (Okuro, Matos Júnior, & Favaro-Trindade, 2013).

A wide variety of actives have been incorporated into microparticles produced by spray chilling. Some applications include functional ingredients like probiotic microorganisms and prebiotics (Okuro, Thomazini, Balieiro, Liberal, & Fávaro-Trindade, 2013; Pedroso, Dogenski, Thomazini, Heinemann, & Favaro-Trindade, 2013), flavors (Sillick & Gregson, 2012) and vitamins and minerals

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(Dubey & Windhab, 2013; Wegmuller, Zimmermann, Buhr, Windhab, & Hurrell, 2006), among others.

Lipid matrices have been used to encapsulate phenolic compounds like curcumin (Guri, Gulseren, & Corredig, 2013), resveratrol (Neves, Lúcio, Martins, Lima, & Reis, 2013) and rosmarinic acid (Campos, Madureira, Gomes, Sarmento, & Pintado, 2014) into nanostructured lipid carriers (NLC) and solid lipid nanoparticles (SLN) by techniques such as hot melt homogenization and others. Currently, there are not works reporting on the application of spray chilling technique for phenolics encapsulation in lipid materials yet. Many types of lipid materials have been used as carrier agents to produce microparticles by spray chilling. Most of them are obtained from modifications (interesterification or hydrogenation) of vegetable oils, like palm and palm kernel. The use of fatty acids like stearic, oleic and lauric, or blends among them, represents another choice that can supply good results (Chambi, Alvim, Barrera-Arellano, & Grosso, 2008; Ribeiro, Arellano, & Grosso, 2012; Sartori, Consoli, Hubinger, & Menegalli, 2015), but it can increase the cost of the product, since fatty acids are more expensive than vegetable oils. Fully hydrogenated soybean oil (FHSO) was successfully used by Guo, Chan, and Heng (2005) and Gamboa, Gonçalves, and Grosso (2011) as carrier agent to produce microparticles containing aspirin and α-tocopherol, respectively. In order to obtain materials with lower melting points, adding soybean oil to FHSO may be an interesting proposal for application as carrier agent in spray chilling. This is also an economic alternative, since soybean oil is the second oil most produced in the world (Gunstone, 2013), and thus highly available.

Gallic acid is a phenolic acid considered a natural antioxidant. This phenolic and its derivatives are applied in food, pharmaceuticals and cosmetics to prevent rancidity induced by lipid peroxidation (Yen, Duh, & Tsai, 2002). Spray drying (Medina-Torres et al., 2013; Robert, Garcia, Reyes, Chavez, & Santos, 2012) and complex coacervation (Lam et al., 2012) techniques have also been used for microencapsulation of this compound.

This study had as main goal the investigation of spray chilling technique to produce solid lipid microparticles (SLMs) loaded with gallic acid as a model phenolic compound. Blends composed of soybean oil and fully hydrogenated soybean oil were evaluated as wall materials. The best conditions for emulsion preparation were studied and microparticles obtained were characterized by gallic acid superficial concentrations, encapsulation efficiencies, mean diameter and size distribution and microstructure.

2. Material and methods

2.1. Material

Gallic acid was purchased from Sigma Aldrich (São Paulo, SP, Brazil) and was used as active material. Refined soybean oil (SO) was acquired commercially in Campinas (SP, Brazil). Fully hydrogenated soybean oil (FHSO) was supplied by Triângulo Alimentos (Itápolis, SP, Brazil). The emulsifier polyglycerol polyricinoleate (Grinsted® PGPR Super) was kindly donated by Danisco Brasil Ltda (Cotia, SP, Brazil). All other reagents were of analytical grade.

2.2. Melting point of lipid blends

Lipid blends with FHSO/SO proportions 60/40, 70/30, 80/20 and 90/10 were assessed as carrier agents. Their thermal behavior was performed by Differential Scanning Calorimetry (DSC), in a thermal analyzer Modulated DSC-2920 (TA Instruments – New Castle, De, USA), according to the AOCS procedure Cj 1-94 (AOCS, 2004). Hermetic aluminum pans containing about 5 mg of each sample were heated up to 80 °C and equilibrated at this temperature for 5 min, and then cooled down to −40 °C (10 °C/min) for 30 min. After that, samples were heated again to 80 °C (5 °C/min). Melting curves were obtained from the second heating. Data were processed by the TA Universal Analysis software. Results are shown in Table 1.

2.3. Defining emulsion preparation conditions

The dispersion of a gallic acid aqueous solution into lipid blends characterizes the formation of water-in-oil (W/O) emulsions. In order to produce kinetically stable emulsions for atomization in spray chilling, a three step experimental plan was designed, in which emulsions kinetic stability was evaluated as response.

Kinetic stability (measured as described in 2.3.2) was used as the indicator parameter to define the value of the variables in each of the three experimental steps. At first, emulsions with PGPR concentrations of 0, 4 and 8 g/100 g lipid blend were prepared by stirring at 11,500 rpm for 8 min. The selected PGPR concentration was further used in the two subsequent steps. To determine the time of agitation, emulsions were stirred at 11,500 rpm for 4, 8 or 12 min. Finally, stirring speeds of 9,500; 11,500 and 14,500 rpm were tested, with the time of agitation previously selected.

2.3.1. Emulsions preparation

In this part of the study, emulsions continuous phases were formed by an 80/20 (FHSO/SO) blend. The disperse phase was a gallic acid aqueous solution, which was prepared as follows: gallic acid was first dispersed in an amount of ethanol corresponding to 5 g/100 g of the solution, and then completed with distilled water at 60 °C, under magnetic stirring, to reach the concentration of 60 g/L. Once the compound was completely dissolved, the solution was heated up to 70 °C and then added to the lipid blend (continuous phase). This last heating was done to avoid lipid crystallization.

After FHSO was completely melted, soybean oil and PGPR, which had been preheated up to 70 °C, were added. This mixture was magnetically stirred for 1 min (IKA Magnetic Stirrer basic HR 1, Janke and Kunkel, Staufen, Germany). Gallic acid solution was then added, maintaining agitation. After 1 min, a rotor–stator device (Ultra Turrax T10 basic, IKA, Janke and Kunkel, Staufen, Germany) was used to homogenize the emulsion with speed and stirring time varying according to the assay.

Each emulsion was prepared with a constant W/O mass ratio of 30/70, in an amount of 25 g in 50 mL-reactors with temperature maintained at 79 °C (about 5 °C above lipid blend melting temperature) by a temperature controlled water bath (Quimis Q214M2 – Diadema, SP, Brazil).

2.3.2. Emulsions kinetic stability

Immediately after preparation, emulsions were completely transferred to cylindrical tubes (internal diameter = 17 mm), which were sealed and kept for 4 h at 79 °C. Since in the spray chilling process used in this work the time comprised between emulsion preparation and atomization did not exceed 30 min, evaluating kinetic stability for 4 h was considered sufficient for determining preparation conditions of emulsions. Aqueous phase separation was measured for all trials and expressed as sedimentation index (SI), which was calculated as a percentage of the ratio between aqueous phase height (H), and the total height (H_T), as shown in Equation (1).

$$SI = \frac{H}{H_T} * 100 \quad (1)$$

Table 1
Melting point of lipid blends and viscosity (η) of lipid blends and W/O emulsions immediately after preparation. Different lowercase letters in each column represent statistically significant difference ($p \leq 0.05$).

FHSO/SO ratio	Lipid blends			Emulsions		
	Melting point (°C)	η (Pa.s)	R^2	W/O ratio	η (Pa.s)	R^2
60/40	71.30 ± 0.47	0.0185[a]	0.9999	30/70	0.0322[c]	0.9998
				20/80	0.0254[a]	0.9999
70/30	74.31 ± 1.01	0.0190[b]	0.9999	30/70	0.0334[cd]	0.9998
				20/80	0.0267[ab]	0.9999
80/20	74.65 ± 1.01	0.0200[c]	0.9999	30/70	0.0335[cd]	0.9997
				20/80	0.0265[ab]	0.9999
90/10	74.99 ± 1.26	0.0207[d]	0.9999	30/70	0.0342[d]	0.9999
				20/80	0.0283[b]	0.9999

2.4. Production of solid lipid microparticles containing gallic acid

Eight formulations of W/O emulsions were used for SLMs production. They differed from each other by the lipid blend (60/40, 70/30, 80/20 and 90/10 FHSO/SO) and by the W/O mass ratio (30/70 and 20/80). Samples preparation was performed as described in 2.3.1 (Emulsions preparation), with the parameters then selected. A Mini Spray Dryer B-290 Büchi (Flawil, Switzerland) was used to produce microparticles. Emulsions, which were kept at approximately 5 °C above lipid blends melting points, were fed into a double fluid atomizer with a nozzle diameter of 0.7 mm by a peristaltic pump (Rex Engineering – Miami, Fla., USA), at a 0.530 L/h flow rate. Cooling air and atomizing air flows were 35,000 L/h and 667 L/h, respectively. In such conditions, inlet and outlet cooling air temperature were around 7 and 11 °C, respectively. After production, microparticles were stored in hermetically sealed plastic containers and kept at 5 °C. All characterization analyses were performed one day after obtaining microparticles.

2.5. Rheological characterization

Lipid blends and emulsions used for SLMs production had their flow curves determined in a stress-controlled rheometer (Physica MCR301, Anton Paar, UK) at 79 °C, with a cone-plate geometry (d = 50 mm). Each assay was performed in sequential three steps: up-down-up, in which the shear rate varied between 0 and 300 s^{-1}. Samples were analyzed immediately after preparation. The Newtonian model (Equation (2)) was fitted to the results obtained from the second up curve:

$$\sigma = \eta\dot{\gamma} \tag{2}$$

in which σ is the shear stress, η is the viscosity and $\dot{\gamma}$ is the shear rate.

2.6. Characterization of solid lipid microparticles containing gallic acid

2.6.1. Particle size distribution

Particle size distribution was measured using a laser light diffraction instrument, Mastersizer 2000 (Malvern Instruments, Malvern, UK). A small powder sample was suspended in a Tween 20 0.4 g/100 g aqueous solution at about 5 °C, and then added into the dispersion unit of the equipment, which was kept under constant stirring at 1,750 rpm and filled with the same Tween 20 solution. The surfactant was used in order to enable the dispersion of the lipid microparticles in aqueous medium. Particles size was calculated with Fraunhofer approximation, and was given as the mean volumetric size $D_{[4,3]}$(De Brouckere mean diameter – Equation (3) – Fan and Zhu (1998)). Size distribution was characterized by D(0.1), D(0.5) and D(0.9), which represent the diameter of accumulated distribution of 10%, 50% and 90% of total particles. The Span value, that gives the width of size distribution, was calculated from Equation (4) (Jinapong, Suphantharika, & Jamnong, 2008). Each sample was analyzed in triplicate.

$$D_{4,3} = \frac{\sum_{i=1}^{n} n \cdot d_i^4}{\sum_{i=1}^{n} n \cdot d_i^3} \tag{3}$$

in which d_i and n represent particles diameter and number of particles, respectively.

$$Span = \left(\frac{D(0.9) - D(0.1)}{D(0.5)}\right) \tag{4}$$

2.6.2. Total gallic acid (TGA)

Total gallic acid was recovered from microparticles according to the methodology proposed by Maschke et al. (2007), with some modifications. Chloroform (10 mL) was added to ~200 mg of microparticles. The mixture was vortexed for 10 s and left to settle for 10 min. This sequence was repeated and then 10 mL of distilled water was added to the mixture, which was vortexed for 1 min and left to settle for 10 min, twice. Tubes were then centrifuged at 10,000 rpm for 10 min. Gallic acid concentration was determined in the resulting aqueous fraction, as described in 2.6.4. Microparticles formulated with each of the four lipid blends, but without the addition of gallic acid, were produced by the same process described in 2.4 and subjected to the procedure here explained. They were used as control samples, in order to verify if lipid blend composition could affect gallic acid quantification in microparticles.

2.6.3. Superficial gallic acid (SGA)

Sample preparation was based on the methodology described by Ribeiro et al. (2012), with some modifications. An orbital shaker (Tecnal TE-420, Piracicaba, SP, Brazil) was used to mix approximately 250 mg of microparticles with 10 mL of a Tween 80 0.1 g/100 g aqueous solution for 60 s at 100 rpm. The contents were filtered through filter paper (Whatman – qualitative grade 1- no. 1001150). Gallic acid concentration was determined in the resulting aqueous fraction, as described in 2.6.4.

2.6.4. Determination of gallic acid

Gallic acid content of microparticles was quantified using the Folin–Ciocalteau colorimetric method (Swain & Hillis, 1959). Samples obtained as described in 2.6.2 and 2.6.3 were diluted in distilled water in the proportion of 1:10 (4 mL) and then mixed with 0.2 mL of the Folin reagent and 0.6 mL of a sodium carbonate aqueous solution at 200 g/L. After 2 h of incubation at room temperature, the absorbance was read at 765 nm in spectrophotometer (SQ-2800 UV/VIS, UNICO, United Products & Instruments Inc., USA). A calibration curve ($R^2 = 0.9981$) was prepared with concentrations

of gallic acid ranging from 50 to 750 mg/L. Results were expressed as mg of gallic acid/mg of sample and were further used to calculate the encapsulation efficiency.

2.6.5. Encapsulation efficiency (EE)

Encapsulation efficiency (Equation (5)) was expressed as the difference between total and superficial gallic acid concentrations, in relation to the amount of gallic acid added in the emulsions before atomization in spray chilling.

$$EE(\%) = \left(\frac{TGA - SGA}{EGA}\right)*100 \quad (5)$$

in which TGA, SGA and EGA are the total, superficial and emulsion gallic acid concentrations.

2.6.6. Microstructure

Morphology of lipid microparticles was evaluated in a Scanning Electron Microscope (SEM) (LEO Electron Microscopy, model Leo 440i, Oxford–Cambridge, England) with an energy dispersive X-ray (EDX) detector. Acceleration voltage equal to 5 kV and beam current of 50 pA were used. Before analysis, microparticles were covered with a gold layer by a Sputter Coater Polaron (VG Microtech, model SC7620, Uckfield, England).

2.7. Statistical analysis

Data were statistically analyzed by Tukey test using the Statistic 12 software (Statsoft, Tulsa, Okla., USA). Differences between means were considered significant at a 95% confidence level ($p \leq 0.05$).

3. Results and discussion

3.1. Defining emulsions preparation conditions

In the spray chilling process here studied, emulsions should be atomized immediately after preparation, in order to minimize gallic acid exposure to high temperature (79 °C). For that, it is necessary to maintain emulsions kinetically stable at least during the period of atomization, which varies according to the amount of sample and the feed rate into the atomizer.

Sedimentation index of emulsions formulated without PGPR was significantly higher ($p \leq 0.05$) than those with concentrations of 4 and 8 g PGPR/100 g lipid blend, as can be seen in Fig. 1A. This occurrence enhances the need of the emulsifier for this system. Within the same period, emulsions containing 4 and 8 g PGPR/100 g lipid blend had similar ($p \leq 0.05$) sedimentation indices. Thus, the effect achieved in terms of emulsion kinetic stability with 4 g PGPR/100 g lipid blend is similar to that given by twice the concentration. Therefore, PGPR concentration selected for work continuity was set at 4 g PGPR/100 g lipid blend. This concentration is lower than the optimal value of 10 g PGPR/100 g lipid that was found by Benichou, Aserin, and Garti (2001), which evaluated the production of W/O emulsions composed by 30% of aqueous phase, with different oily phases. In the present work, the presence of ethanol and gallic acid in the aqueous phase has probably influenced on obtaining kinetically stable emulsions with a lower concentration of PGPR. These components may have acted as cosurfactants in the system studied, i.e., they may have exerted surface activity on the droplets interface, which is characteristic of compounds such as alcohols and organic acids (Lawrence & Rees, 2012).

Previous studies regarding gallic acid incorporation into emulsions have shown that this compound may present low superficial activity on droplets interface, due to its high polarity, that promotes its partition most to the aqueous phase. However, despite its low superficial activity, it has been found that gallic acid was effective on obtaining olive oil-in-water emulsions with higher stability indices, compared to control emulsions formulated without the addition of the compound. Such effect was attributed to mechanisms like electrostatic repulsion (Di Mattia, Sacchetti, Mastrocola, & Pittia, 2009; Di Mattia, Sacchetti, Mastrocola, Sarker, & Pittia, 2010).

Up to 3 h after emulsion preparation, there was no significant difference ($p \leq 0.05$) between sedimentation indices of samples stirred for 4, 8 or 12 min (Fig. 1B). Therefore, stirring time has not influenced significantly on emulsions kinetic stability within the evaluated range. In order to have a shorter preparation period, stirring time of 4 min was set for the continuity of this work.

Stirring speed has not affected emulsions sedimentation index significantly ($p \leq 0.05$) within the evaluated range (Fig. 1C). Thus, as an alternative to lower energy consumption, stirring speed of 9,500 rpm was chosen for this system. PGPR has the ability to cause a high reduction on interfacial tension when forming W/O emulsions. The lower the interfacial tension, the smaller the energy required to achieve a given droplet size (Scherze, Knoth, & Muschiolik, 2006; Ushikubo & Cunha, 2014). This may be related to the fact that stirring speeds within the range reported in this work have not present a significant effect ($p \leq 0.05$) on emulsions kinetic stability, since the lowest stirring speed used seems to be sufficient for emulsion stabilization.

3.2. Rheological characterization

Flow curves of lipid blends and emulsions are presented in Fig. 2, and viscosity values for those samples are outlined in Table 1. For all samples, there was no yield stress, and shear stress responded linearly to shear rate increasing, thus characterizing a Newtonian flow behavior. Lipid blends viscosity slightly increased with higher FHSO concentrations. Edible oils are Newtonian fluids that present high viscosity, because of the long chain structure of their molecules. The higher the molecule chain the higher the viscosity of the oil (Muller, 1973). Correlation between edible oils viscosity and their composition has been also investigated by many authors (Kim, Kim, Lee, Yoo, & Lee, 2010; Toro-Vazquez, Charó-Alonso, & Pérez-Briceño, 1999; Yalcin, Toker, Ozturk, Dogan, & Kisi, 2012). In general, higher concentrations of unsaturated carbon chains, especially the double unsaturated ones, imply in lower viscosity. This is attributed to the molecular structure of carbon chains, since the presence of the double bonds impair fatty acids molecules to get a highly packed organization, giving the oil a more fluid-like consistency (Kim et al., 2010). Regarding W/O emulsions, shear stress also presented linear dependence to shear rate in 30% W/O emulsions with soybean oil stabilized with PGPR at 25 °C produced by Ushikubo and Cunha (2014).

The incorporation of gallic acid aqueous solutions into the lipid blends resulted in increased viscosity values, which were significantly higher ($p \leq 0.05$) in formulations with the highest W/O ratio (30/70). Water phase proportion in emulsions had higher influence on flow behavior than composition of lipid blend. This can be observed from results presented in Fig. 2, in which flow curves of emulsions and lipid blends are clearly divided into three groups: the first one is composed by the lipid blends and presented the smallest slopes of the three groups; the intermediate group is composed by emulsions formulated with different lipid blends but the same W/O ratio (20/80); and the last group is the one with the highest slopes, in which emulsions were composed of different lipid blends and the same W/O ratio (30/70).

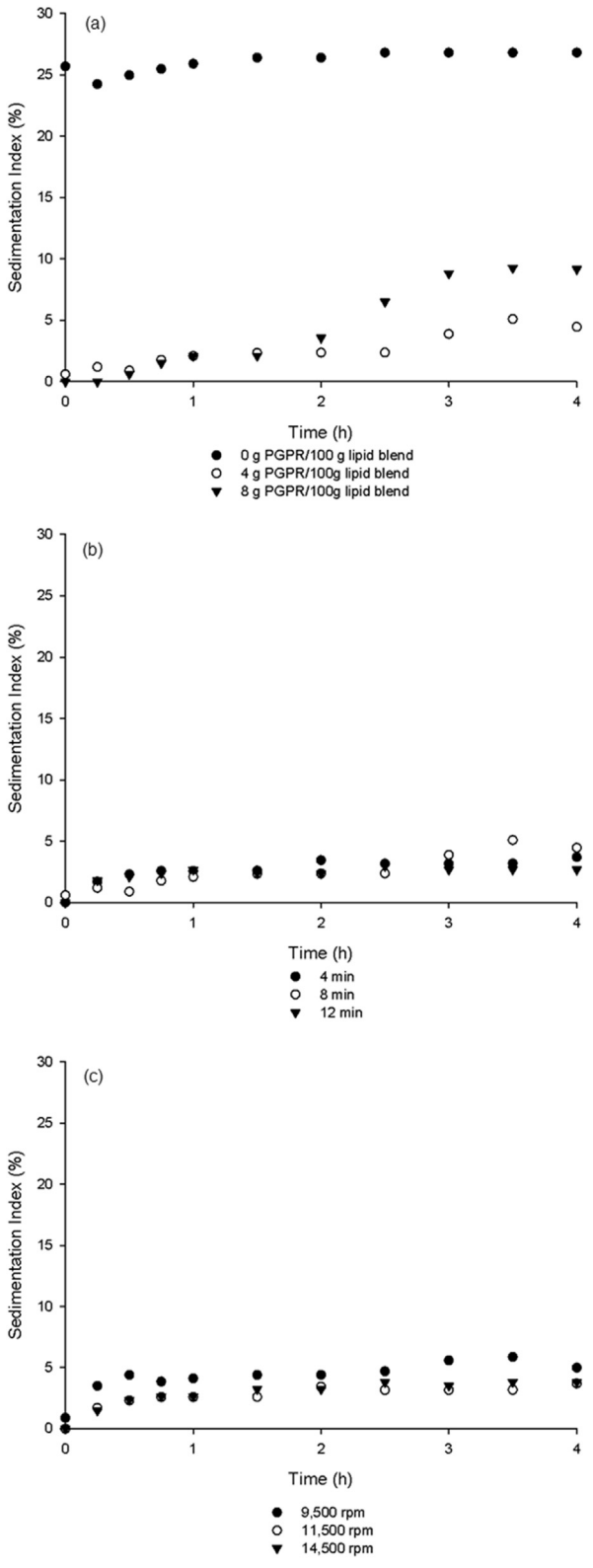

Fig. 1. Emulsions sedimentation index at 79 °C with time. Oil phase was a lipid blend 80/20 (FHSO/SO). Aqueous phase was a gallic acid solution 60 g/L at 60 °C. W/O ratio was 30/70. **(A)** PGPR concentration varying from 0 to 8 g/100 g lipid blend. Stirring speed was kept at 11,500 rpm for 8 min. **(B)** Time for stirring ranging from 4 to 12 min. Stirring speed was kept at 11,500 rpm. PGPR concentration was 4 g/100 g lipid blend. **(C)** Stirring speed ranging from 9,500 to 14,500 rpm. Time of stirring was kept at 4 min and PGPR concentration was 4 g/100 g lipid blend.

3.3. Characterization of lipid microparticles containing gallic acid

3.3.1. Encapsulation efficiency (EE)

The increase of the W/O ratio in emulsions formulations has not affected significantly ($p \leq 0.05$) gallic acid superficial concentration on microparticles. On the other hand, these values were lower in most of the formulations with higher concentrations of FHSO, as shown in Table 2.

Factors influencing superficial content of gallic acid also affected encapsulation efficiency (Table 3), since higher concentrations of core material on microparticles surface imply in lower retention of the material within them. Thus, the highest encapsulation efficiencies were obtained in microparticles formulated with higher FHSO concentrations, for 20/80 and 30/70 W/O ratios.

Studies published by Jores, Mehnert, and Mäder (2003) and by Hu et al. (2005) have reported that in nanostructured lipid carriers formulated with blends composed of high and low melting point lipids, the portion of liquid lipid might get distributed over particles surface. This would happen because the regular structure of the crystals from the high melting point lipid would not be able to accommodate the liquid oil molecules. So, the oil would settle on the outside of the lipid matrix during crystallization of the high melting point lipid. In formulations containing higher concentrations of soybean oil, the W/O interface of droplets in emulsions has an increased mobility due to the presence of higher concentrations of unsaturated fatty acids. In these conditions, gallic acid migration through the W/O interface is favored, which causes it to accumulate in the oily phase of emulsions. In this case, when emulsions are atomized to form the solid microparticles inside the cooling chamber of the spray chiller, the expulsion of the liquid lipid fraction to microparticles surface during crystallization can carry also the core material with it. This would explain the increase on gallic acid superficial concentrations in formulations with higher SO concentrations here found.

3.3.2. Mean diameter and size distribution

Microparticle diameter is a very important characteristic to be assessed, since it can influence on sensorial aspects of products in which microparticles are added (McClements, 2005).

Table 4 presents microparticles mean diameters and size distribution. Lipid blends composition was the factor that most affected particle diameter and size distribution. There was a trend in diameter decreasing in response to the increasing concentration of FHSO, although many samples have presented statistic similar diameters. Mean volumetric diameter $D_{[4,3]}$ decreased from 35.98 μm to 23.91 μm when FHSO mass concentration was increased from 60% to 90%, respectively. This trend could also be noticed in the D_{10}, D_{50} and D_{90} parameters, displayed in Table 4.

The W/O ratio of emulsions has not caused any significant change neither in the mean volumetric diameter $D_{[4,3]}$ nor in the D_{10}, D_{50} and D_{90} parameters, when considering particles formulated with the same lipid blends.

Differently from the results for diameters values, formulation composition has not shown any effect on size distribution width, since microparticles obtained from all formulations presented a small range of span values (1.648–1.905), which were statistically similar among all formulations. This occurrence can also be noticed in Fig. 3, where particle size distribution of samples formulated with 20/80 and 30/70 W/O ratio is shown. A bimodal distribution, which presents two distinct peaks, was observed for the SLMs, indicating two predominant sizes. The first peak had lower volume (<1%) and lower diameter sizes (between approximately 0.5 and 5 μm) for all formulations. The influence of the lipid blends composition on size distribution can be more clearly noticed on the second peak, that presented larger volume (~8–9%) and larger

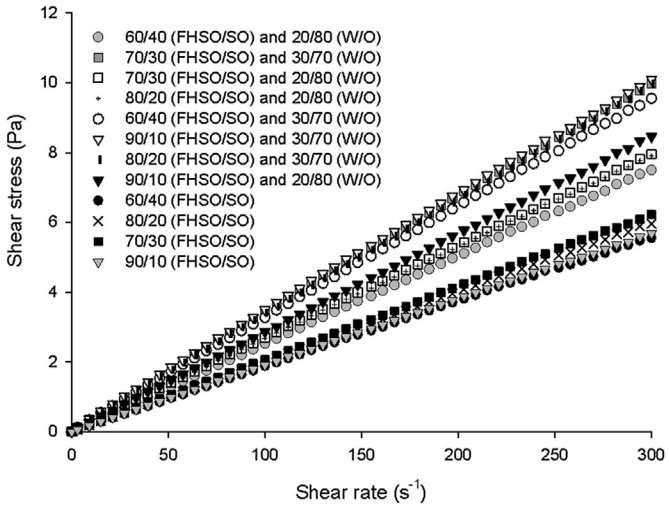

Fig. 2. Emulsions and lipid blends flow curves. Samples were analyzed immediately after preparation, at 79 °C.

Table 2
Gallic acid superficial content on lipid microparticles. Values are expressed as percentage of the theoretical concentration of gallic acid.

FHSO/SO ratio	W/O ratio	
	30/70	20/80
60/40	33.89 ± 4.12^{aC}	38.87 ± 5.68^{aAB}
70/30	55.46 ± 3.73^{bD}	44.68 ± 4.78^{aB}
80/20	22.57 ± 1.17^{aB}	33.98 ± 3.58^{bA}
90/10	11.96 ± 1.75^{aA}	16.04 ± 3.26^{bC}

Values are averages of two experiments, with each individual sample analyzed in triplicate. Different lowercase letters in each row and different capital letters in each column represent statistically significant difference ($p \leq 0.05$).

Table 3
Encapsulation efficiency of lipid microparticles. Values are expressed as percentage of the theoretical concentration of gallic acid.

*FHSO/SO ratio	W/O ratio	
	30/70	20/80
60/40	74.67 ± 1.19^{bB}	62.59 ± 9.80^{aAB}
70/30	54.14 ± 2.82^{aA}	55.16 ± 4.96^{aA}
80/20	83.48 ± 6.48^{aC}	71.90 ± 11.47^{aBC}
90/10	101.83 ± 6.74^{bD}	80.01 ± 2.76^{aC}

Values are averages of two experiments, with each individual sample analyzed in triplicate. Different lowercase letters in each row and different capital letters in each column represent statistically significant difference ($p \leq 0.05$).
*Gallic acid concentrations in microparticles formulated with the 90/10 lipid blend were corrected, considering the effect caused by this lipid blend on the absorbance reading of the samples in the Folin–Ciocalteau method. For samples formulated with the other three lipid blends, this effect was not detected.

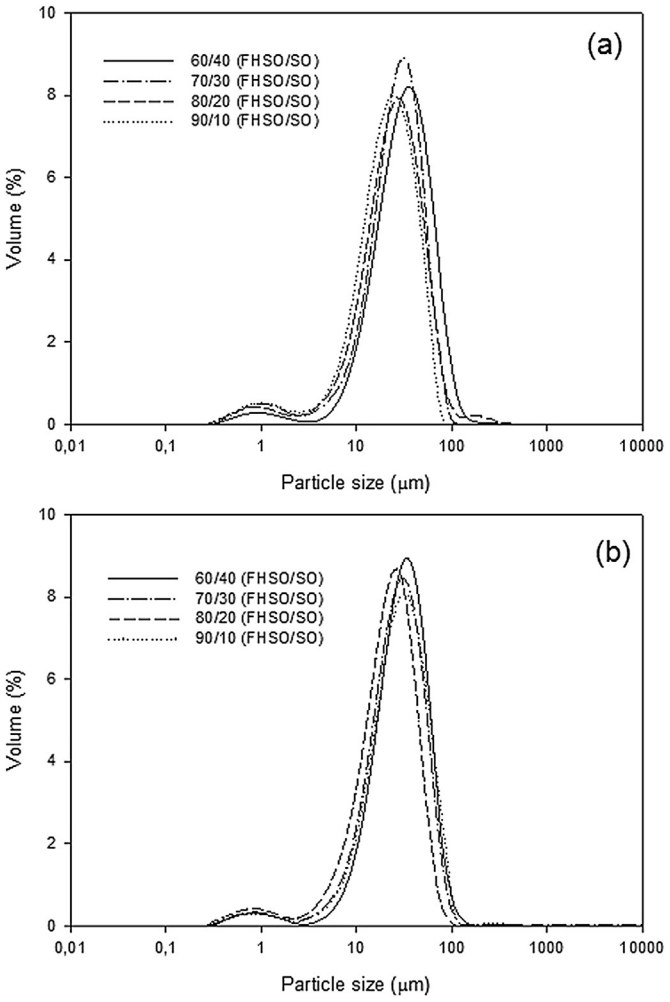

Fig. 3. Particle size distribution of microparticles produced with different lipid blends at **(A)** 20/80 and **(B)** 30/70 W/O mass ratio.

particle size (~20–30 μm). Microparticles formulated with lower FHSO concentrations had the second peak slightly shifted to the right, especially in Fig. 3A, indicating an increased mean diameter. Although span values and D_{10}, D_{50} and D_{90} parameters presented similar values for microparticles formulated with 20/80 or 30/70 W/O ratios, size distribution in Fig. 3 has shown more homogeneous particle sizes at a 30/70 W/O ratio. The higher lipid concentration of the other formulations may have caused particle agglomeration, due to lipid coalescence, thus resulting in a more heterogeneous size distribution.

The crystallization of liquid lipids causes volume to become

Table 4
Mean diameter ($D_{[4,3]}$) and size distribution of lipid microparticles.

FHSO/SO ratio	W/O ratio	$D_{[4,3]}$ (μm)	Span	$D_{(0.1)}$ (μm)	$D_{(0.5)}$ (μm)	$D_{(0.9)}$ (μm)
60/40	30/70	34.30 ± 5.48^{bd}	1.648 ± 0.170^{a}	11.59 ± 1.35^{c}	30.01 ± 2.80^{cd}	61.41 ± 10.67^{ac}
	20/80	35.98 ± 2.17^{d}	1.749 ± 0.122^{a}	11.85 ± 1.17^{c}	31.42 ± 2.37^{d}	66.64 ± 3.97^{c}
70/30	30/70	30.60 ± 3.99^{abd}	1.778 ± 0.210^{a}	9.15 ± 1.53^{b}	26.61 ± 2.23^{ac}	56.42 ± 6.93^{abc}
	20/80	26.68 ± 2.29^{ac}	1.684 ± 0.247^{a}	7.23 ± 0.96^{ab}	24.77 ± 1.81^{ab}	48.88 ± 6.49^{ab}
80/20	30/70	28.39 ± 2.35^{abc}	1.798 ± 0.214^{a}	8.29 ± 1.19^{ab}	25.45 ± 2.10^{ab}	53.58 ± 5.25^{ab}
	20/80	32.13 ± 3.44^{abd}	1.905 ± 0.107^{a}	8.57 ± 1.44^{ab}	26.40 ± 3.23^{ac}	58.76 ± 6.96^{ac}
90/10	30/70	28.74 ± 3.17^{abc}	1.761 ± 0.337^{a}	8.70 ± 1.41^{ab}	25.26 ± 1.37^{ab}	53.17 ± 8.10^{ab}
	20/80	23.91 ± 1.60^{c}	1.812 ± 0.106^{a}	6.54 ± 0.76^{a}	21.35 ± 1.22^{b}	45.33 ± 3.62^{b}

Values are averages of two experiments, with each individual sample analyzed in triplicate. Different lowercase letters in each column represent statistically significant difference ($p \leq 0.05$).

more compact. The inverse process, melting, leads to volume expansion (Tan & Che Man, 2002). FHSO is a high melting point lipid, due to its high concentration of saturated fatty acids like stearic acid and palmitic acid, which represent 87% and 11% in mass, respectively, of the total fatty acids present in this lipid (results for fatty acid composition not shown here). Because of this characteristic, FHSO is completely crystallized at room temperatures (~25 °C), whereas vegetable oils such as soybean oil are in the liquid state. In this work, decreased mean volumetric diameters found in microparticles formulated with higher FHSO concentrations may be related to the shrinkage in their volume, since the major lipid fraction, in that case, is completely crystallized. The reduction of SLM mean diameter with higher concentrations of stearic acid was also reported by Ribeiro et al. (2012) and by Alvim, Souza, Koury, Jurt, and Dantas (2013).

3.3.3. Microparticles microstructure

Microparticles of spherical shape and smooth surface were obtained from all formulations. As indicated by the arrows in Fig. 4A and F, there are few crystals located on microparticles surface, which are apparently formed by the lipid wall material.

The presence of orifices can be noticed on the surface of some microparticles, despite their continuous shell. Such orifices may be caused by the formation of air bubbles inside the lipid matrix, due to air incorporation during emulsion homogenization. They are highlighted by the arrows in Fig. 4C, E and H.

Particle agglomerates were formed in all samples. Those with smaller diameters were adhered to the surface of the ones with higher sizes, as indicated in Fig. 4B and G. Besides agglomeration of whole particles, merging microparticles were also observed (Fig. 4E and F).

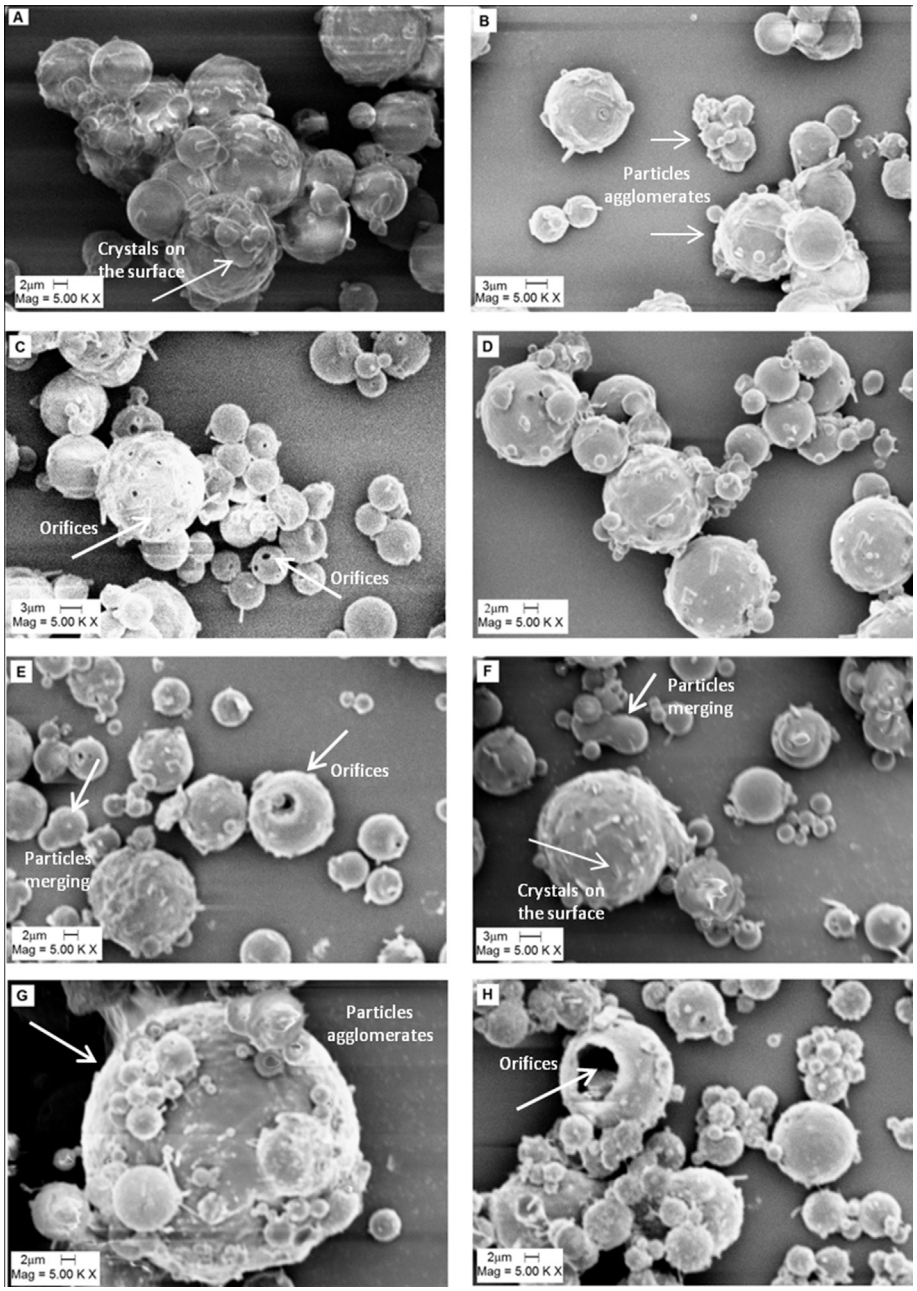

Fig. 4. Micrographs obtained by SEM of lipid microparticles containing gallic acid. Letters in each picture represents FHSO/SO and W/O proportions in formulations. **(A)** 60/40, 70/30; **(B)** 60/40, 80/20; **(C)** 70/30, 70/30; **(D)** 70/30, 80/20 **(E)** 80/20, 70/30; **(F)** 80/20, 80/20; **(G)** 90/10, 70/30; (H) 90/10, 80/20.

According to Shi, Liang, and Hartel (2005), crystal morphology in lipid mixtures can be dominated by high melting point lipids. Thus, in the present work the presence of crystals on microparticles surface may be related to the high concentration of stearic acid from FHSO. This observation is close to the results presented by Rodriguez et al. (1999) and Chambi et al. (2008). In both works, imperfections and wrinkles on microparticles surface produced by techniques similar to spray chilling were related to the presence of stearic acid in formulations.

The occurrence of fat crystals and particle agglomerates was also reported by Wegmuller et al. (2006). According to the authors, agglomeration may occur due to microparticles which are not completely solidified when they reach the bottom of the cooling chamber. About the fat crystals on microparticles surface, the authors observed that such crystals had lower frequency in particles with smaller diameter, because they require shorter time to be fully solidified.

4. Conclusions

The use of spray chilling technique for production of lipid microparticles containing gallic acid is a good alternative when using the soybean oil matrix proposed in this work. Higher concentrations of FHSO led to increased encapsulation efficiencies, making it possible to obtain values equal or greater than 80% in the best formulations. Composition of the lipid blend was the main factor influencing particle size, which has decreased in formulations with higher FHSO concentrations. Further investigations on release kinetics are necessary, in order to employ the lipid microparticles here studied as gallic acid carriers in food and pharmaceutical products.

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