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Physicochemical characterization and stability of chia oil microencapsulated with sodium caseinate and lactose by spray-drying

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ABSTRACT

Microencapsulation is an alternative to protect chia oil from the adverse influence of the environment, since this oil is susceptible to oxidation due to their high content of polyunsaturated fatty acids (PUFAs). The aim of this study was to investigate the influence of the operating conditions (homogenization pressure for emulsifying and spray-drying inlet/outlet temperatures) on the physicochemical properties of chia oil microencapsulated with sodium caseinate and lactose by spray-drying. Oil-in-water emulsions were prepared with 10 %wt/wt sodium caseinate, 10 %wt/wt lactose and 10 %wt/wt chia oil using a high pressure homogenizer at 400 and 600 bar. These emulsions were spray-dried at inlet/outlet temperatures of 135/70 °C and 170/90 °C. The microcapsules were approximately spherical in shape with some small pores and a polydisperse particle size distribution, recording moisture contents of 1.48–3.52 g/100 g (d.b.). The encapsulation efficiency was >90% in all cases. The particle size distributions of parent and reconstituted emulsions displayed a unimodal distribution. The emulsion reconstituted of microcapsules obtained from emulsions at 400 bar presented a higher initial particle size (D [4,3] = 0.48–0.54 μm; D [3,2] = 0.31–0.33 μm) than those prepared at 600 bar (D [4,3] = 0.33–0.35 μm; D [3,2] = 0.24–0.25 μm). The dispersibility of microcapsules was assessed by measuring the change in droplet obscuration as a function of time. Physical stability of the different reconstituted emulsions was studied by recording the backscattering profiles (BS) as a function of the cell length and time. Regarding oxidative stability during storage, chia bulk oil showed higher peroxide values (PV) than microencapsulated oil during storage. The microcapsules obtained from emulsions at 600 bar showed higher PV than those at 400 bar. The results indicate that the microencapsulation process consisting of emulsification using high-pressure valve homogenizer and subsequent spray-drying is suitable for preparing sodium caseinate-lactose-based microcapsules containing chia oil.

1. Introduction

In recent years there has been a development of underexploited promising plant species as a source of dietary or specialty oils. Also, there is an increasing demand for nutritive and healthy foods in the market and this fact has led the food industry to focus their research in products of this nature. Chia (*Salvia hispanica* L.) seed oil is well-known as a good source of polyunsaturated fatty acids (PUFAs), mainly ω-3 (α-linolenic acid ~60%). It contains also a low percentage of saturated fatty acids and other bioactive components such as tocopherols, polyphenols, carotenoids and phospholipids [1,2]. Thus, this oil can be described as "gourmet oil" since it is recognized as high quality oil and appreciated for its flavor, color and healthy characteristics.

Even though the fatty acid profile of chia seed oil is nutritionally favorable, the high degree of unsaturation of ω-3 makes it very susceptible to oxidation. This makes the incorporation of ω-3 fatty acids (FAs) into foods a significant challenge since their susceptibility to lipid oxidation and development of off-flavors affects the sensory properties of ω-3 fortified foods. Microencapsulation is proving to be a successful strategy for protecting sensitive ingredients and enabling their delivery into foods. It has the potential to offer novel solutions for stabilization and improved delivery of ω-3 FAs in foods [3]

Spray-drying is one of the oldest encapsulation methods and the most commonly used in food industry. The general process involves the dispersion of the core material into a polymer solution, forming an emulsion or dispersion, followed by homogenization of the liquid and atomization of the mixture into the drying chamber [4]. This leads to evaporation of the water and the formation of matrix-type microcapsules. For the production of microencapsulated oils with high ω-3 FA content, it is essential to design the formulation and apply the appropriate process to obtain stable liquid emulsions before conversion into

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powder by drying. Processing conditions such as temperature, shear, pH of the emulsion, total solids, drying rate, and order of addition of each of the components used during manufacture of omega-3 oil powders influence the microcapsule properties and stability. Although drying is only one of the steps involved in the production of microcapsules, the control of the processing conditions during drying has a significant influence on the physical properties of the final powder microcapsule [3]. The main factors in spray-drying that affect the final product are feed temperature, air inlet temperature, and air outlet temperature. In order to obtain good microencapsulation efficiency optimal spray-drying conditions must be used [5].

Over the last few years, the main emphasis of microencapsulation of food flavors and oils has concentrated on improving the encapsulation efficiency during spray-drying for extending the shelf-life of the products by minimizing the amount of unencapsulated oil at the surface of powder particles. The properties of wall and core materials as well as the emulsion characteristics and drying parameters are the factors that can affect the efficiency of encapsulation. Emulsification plays a key role in optimizing the encapsulation efficiency of food flavors and oils [6].

Protein and carbohydrate materials are commonly used for microencapsulation of ω-3 FAs and oils. These compounds can be used alone or in combination with other components in the formulation such as oxygen scavengers, antioxidants, chelating agents, and surfactants to achieve certain functions either during production or in the final product [3].

Most of the research work found in the literature about microencapsulation of unsaturated FA-rich oils use fish or flaxseed oils as core material [6–13]. However, very little information is available on microencapsulation of chia seed oil by spray-drying. Rodea-González, Cruz-Olivares, Román-Guerrero, Rodríguez-Huezo, Vernon-Carter and Pérez-Alonso [14] studied the microencapsulation of chia oil using binary biopolymers blends of whey protein concentrate with gum Arabic or mesquite gum. The authors found that encapsulation efficiency was higher than 70% for all microcapsules and that both binary biopolymer blends produced parent and reconstituted emulsions stable against the droplet coalescence.

The aim of this study was to investigate the influence of the operating conditions (homogenization pressure for emulsifying and spray-drying inlet/outlet temperatures) on the physicochemical properties and stability of chia oil microencapsulated with sodium caseinate and lactose by spray-drying.

2. Material and methods

2.1. Materials

Chia seed oil was purchased from SDA S.A. (Lobos, Argentine). It was obtained by cold pressing and stored until use at 4 ± 1 °C in darkness using amber glass bottles without head space. Sodium caseinate (NaCas) from bovine milk was purchased from Sigma Chemical Company (St. Louis, MO) and D-lactose monohydrate from Anedra (Argentine). Reagent grade chemicals were used otherwise. For preparation of solutions, distilled water was used.

2.2. Characterization of chia oil

Fatty acid composition was analyzed by GC according to IUPAC 2.302 standard method. FAMEs were prepared using BF_3-methanol reagent following IUPAC 2.301 method [15].

Iodine and saponification values, refractive index and free fatty acid content were determined according to AOCS recommended practices Cd1c-85, Ca6a-40, Cc7-25 and Ca5a-40, respectively [16].

Oil tocopherol content was determined by normal phase HPLC using a Hewlett Packard chromatography system (HPLC Hewlett Packard 1050 Series, Waldbronn, Germany) equipped with a fluorescence detector Agilent 1100 Series (Agilent Technology, Palo Alto, CA, USA) following the procedures described in IUPAC 2.432 [15] and AOCS Ce8-89 [16].

2.3. Preparation of oil-in-water (O/W) emulsions

Chia seed oil (10 g/100 g emulsion) was blended with aqueous solutions of sodium caseinate (NaCas) (10 g/100 g emulsion) and lactose (10 g/100 g emulsion) using an Ultra-Turrax T25 high-shear probe mixer (Janke & Kunkel GmbH, Staufen, Germany) operated at 10,000 rpm for 60 s to give pre-emulsions. The resultant emulsions were further homogenized at 400–600 bar with four recirculations using a high-pressure laboratory valve homogenizer (Panda 2K, GEA Niro Soavi, Parma, Italy). Nisine (12.5 mg/kg) and potassium sorbate (1000 mg/kg) were added to the emulsions as food grade preservatives. This was necessary because the physical stability of the reconstituted O/W emulsions was studied for a period of 190 h.

2.4. Characterization of parent O/W emulsion

2.4.1. Particle size distribution and mean diameters

Particle size distribution, and De Broucker (D [4,3]) and Sauter (D [3,2]) mean diameters of particles of the emulsions were determined with a particle size analyzer (Malvern Mastersizer 2000E, Malvern Instruments, Worcestershire, UK). About 1 mL of sample was diluted to 600 mL of water and the pump rate in dispersion unit was set at 2000 rpm (Hydro 2000MU, Malvern Instruments, Worcestershire, UK) [17,18]. The relative refractive index (refractive index of chia oil/refractive index of water) of the different emulsions was 1.10.

2.4.2. Rheology

Emulsion viscosity was measured in a Haake RS600 controlled stress oscillatory rheometer (Haake, Karlsruhe, Germany) at 25 ± 0.2 °C using a plate–plate sensor system with a 1.0 mm gap between plates and shear rates from 1.0 to 500 s^{-1}. For non-Newtonian fluids Weissenberg–Rabinowitsch correction was applied to transform the apparent rheometer data to true data [19].

2.4.3. Emulsion stability

The backscattering of light was measured using a Vertical Scan Analyzer Quick Scan (Coulter Corp., Miami, FL, USA), according to Cabezas, Madoery, Diehl and Tomás [18]. The backscattering of monochromatic light (λ = 850 nm) from the emulsions was determined as a function of the height of the sample tube (ca. 65 mm) during a period of 190 h. The basis of the multiple light scattering theory has been exhaustively studied by Mengual, Meunier, Cayré, Puech and Snabre [20].

2.5. Preparation of microcapsules by spray-drying

The emulsions were spray-dried at a feed rate of 0.6 L/h at 135/70 °C or 170/90 °C inlet/outlet temperatures using laboratory scale Buchi spray-drier (Mini Spray dryer B-191, Buchi Labortechnik, Flawil, Switzerland) equipped with 0.5 mm diameter nozzle.

2.6. Storage of microcapsules

About 10 g of encapsulated oil was spread on glass Petri dishes (95 mm × 15 mm) for the storage stability test. Also, about 10 g of bulk oil was placed in 50-mL glass beaker. The dishes and beakers were kept open to allow contact with air, placed in a chamber with saturated $MgCl_2$ solution (RH 33%) at 20 ± 2 °C in the dark. Two desiccators were used to duplicate the assay. The humidity chambers were balanced before the samples were set in. Samples were withdrawn at frequent time intervals for analysis.

2.7. Characterization of the microcapsules

2.7.1. Moisture content

Samples of approximately 2 g of powder were placed in an aluminum pan and dried for 24 h at 70 °C and 29 in Hg in vacuum oven (Instrumentación Científica S.A., Buenos Aires, Argentina). Moisture content was calculated from the weight difference [21].

2.7.2. Water activity (a_w)

The a_w of the microcapsules was determined (Aqua-Lab Water Activity Meter, Series 3, Decagon Devices Inc., USA) at 25.0 ± 0.5 °C.

2.7.3. Microencapsulation efficiency

The procedure to determine the total oil content of microcapsules was based on the Rose–Gottlieb method [22]. The free oil fraction (non-encapsulated oil fraction) was determined according to Sankarikutty, Sreekumar, Narayanan and Mathew [23]. Briefly, hexane (200 mL) was added to 4 g powder and the mixture stirred in a stoppered flask for 15 min at 25 ± 2 °C. The mixture was filtered (Whatman No. 4) and the solvent was evaporated at 40 °C using a rotary vacuum evaporator (Büchi, Flawil, Switzerland). Microencapsulation efficiency (ME %) was calculated from the following equation:

$$ME\% = \left(\frac{TO - FO}{TO}\right) \times 100 \quad (1)$$

where TO is the total oil content in microcapsules and FO is the free oil content determined as previously described.

2.7.4. Scanning electron microscopy (SEM)

Particle size and morphology of microcapsules were analyzed by scanning electron microscopy (SEM). Microcapsules were adhered to a cover slip, coated with a thin gold film (600 Å) in a sputter coater (Pelco 9100, Clovis, CA, USA) and observed in a scanning electron microscope (LEO EVO VP, Cambridge, England) under high vacuum with a 5 kV acceleration voltage.

2.7.5. Dispersibility

Dispersibility of spray-dried emulsions was determined according to Klinkesorn, Sophanodora, Chinachoti, McClements and Decker [24]. Briefly, ~0.3 mg of powder/mL of distilled water was added within the stirring chamber of a laser diffraction instrument (Malvern Mastersizer Model 2000 E, Malvern Instruments, Worcestershire, UK) at 2000 rpm during 5 min. The dispersibility of the powdered emulsion was assessed by measuring the change in mean particle diameter (D [4,3]) and obscuration as a function of time.

2.7.6. Particle size distribution, mean diameter and physical stability of reconstituted emulsions

Spray-dried chia oil powders were reconstituted in distilled water (10 g solids/100 g emulsion) [24] at room temperature (~25 °C) for 30 min under constant magnetic stirring. The reconstituted emulsions were analyzed in the same manner as the parent emulsions (Sections 2.4.1 and 2.4.3).

2.7.7. Peroxide value

Lipid oxidation was evaluated by determination of the peroxide value. The oil was extracted according to the method described by Partanen, Hakala, Sjövall, Kallio and Forssell [25]. A sample of 0.5 g of powder was weighed into a test tube and suspended in 5 mL of distilled water. The tube was shaken until complete powder dissolution. A 300 μL portion was taken and vortexed 3 times for 10 s with 1.5 mL of an isooctane/isopropanol (3:1 v:v) mixture. The phases were separated and the upper phase was taken for analysis. Duplicate extractions were performed. Peroxide value was determined spectrophotometrically, according to Mancuso, McClements and Decker [26] method. A portion of the extraction medium (10–100 μL) was added to 2.8 mL of a methanol/butanol (2:1 v:v), followed by 15 μL of thiocynate solution (3.94 M) and 15 μL ferrous iron (0.072 M acidic solution). The sample was briefly vortexed, reacted in the dark for 20 min, and the absorbance was measured at 510 nm. Lipid hydroperoxide concentrations were determined using cumene hydroperoxide standard curve.

2.8. Statistical analysis

Statistical analysis was performed by ANOVA at 5% significance level ($p \leq 0.05$). Means were separated according to Tukey's multiple comparison tests ($p \leq 0.05$) in all cases. Normal distribution of the variables was checked by the Kolmogorov–Smirnov test ($p \leq 0.05$) and variance check was done by Cochran's test. When it was necessary, data were transformed to avoid the violation of the assumptions underlying the ANOVA test. Data were processed using the Statgraphics Centurion XV.II for Windows software (Statpoint Technologies, Warrenton, VA, USA).

3. Results and discussion

3.1. Characterization of chia oil

The initial characteristics of chia seed oil used in this study are shown in Table 1. These results are within chia seed oil characteristics reported in previous work [1,2].

3.2. Characterization of parent O/W emulsions (PE)

According to Polavarapu, Oliver, Ajlouni and Augustin [27], the preparation of a stable emulsion prior to drying is critical for high microencapsulation efficiency. Small oil droplets will be enclosed and embedded more efficiently within the wall matrix of the microcapsules and also, the resulted emulsion will be more stable during the spray-drying encapsulation process [6]. Volume and surface diameters D [4,3] and D [3,2] of particles were 0.38 ± 0.02 μm and 0.29 ± 0.01 μm, respectively, for emulsions obtained at 400 bar and decreased significantly ($p \leq 0.05$) when the homogenization pressure increased to 600 bar (D [4,3] = 0.23 ± 0.01 μm; D [3,2] = 0.21 ± 0.01 μm). The particle size distribution curves were unimodal for both emulsions and showed a normal shape (Fig. 1a). The results indicated that fine O/W emulsions, necessary for microencapsulation by spray-drying were obtained. The particle size is an important parameter for determining the stability of the emulsion prior to drying and may also affect the characteristics of the final spray-dried microencapsulated powder [4].

The parent emulsions obtained at 400 and 600 bar displayed shear-thinning behavior (Fig. 2), which is a common feature of O/W emulsions [27]. All experimental data of rheological analyses of the homogeneous

Table 1
Physicochemical characteristics of chia seed oil.

Fatty acids (%)	
$C_{16:0}$	8.5 ± 0.9
$C_{18:0}$	2.0 ± 0.6
$C_{18:1}$	5.2 ± 0.6
$C_{18:2}$	19.0 ± 0.6
$C_{18:3}$	65.4 ± 1.4
Iodine value (g I_2/100 g oil)	213.0 ± 3.4
Saponified value (mg KOH/g oil)	193.5 ± 0.1
Refractive index (25 °C)	1.4794 ± 0.0001
Free fatty acids (g oleic acid/100 g oil)	0.6 ± 0.1
Tocopherols (mg/kg oil)	
Total	443 ± 4
α-	6 ± 1
γ-	415 ± 5
δ-	22 ± 1
Peroxide value (meq peroxide/kg oil)	2.0 ± 0.1

Mean values ± standard deviation of two independent batches.

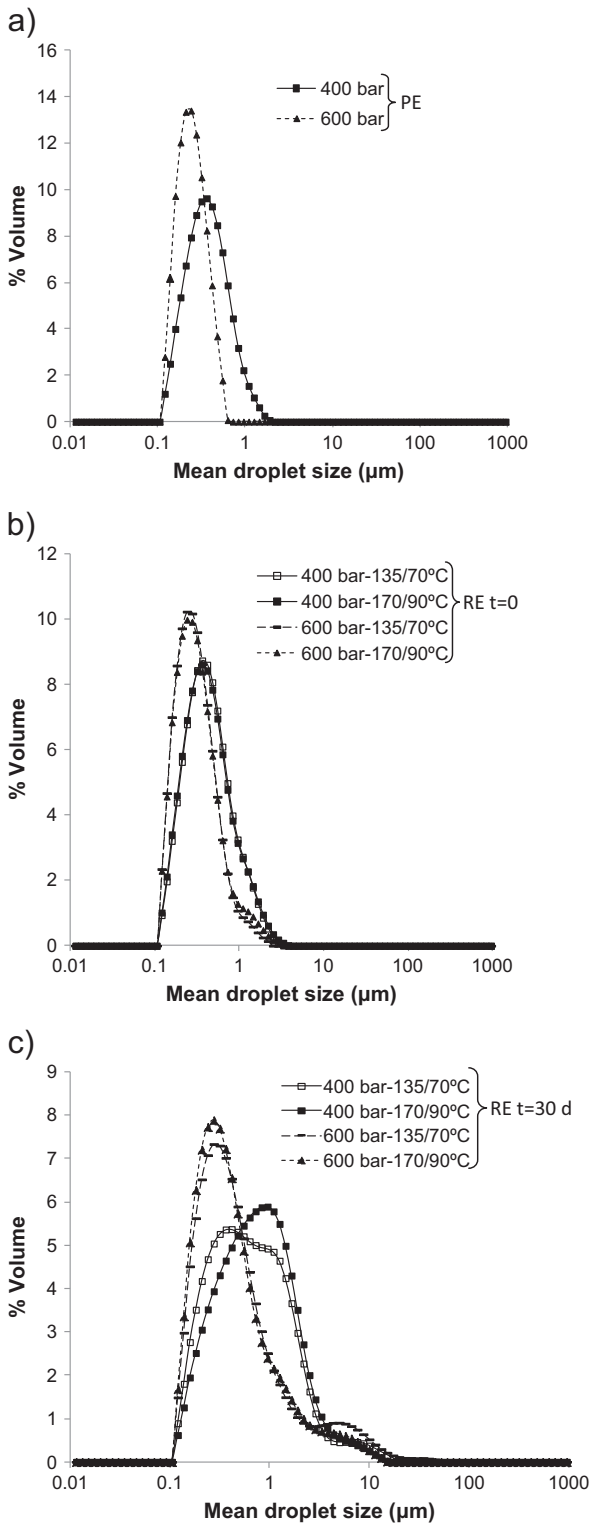

Fig. 1. Volume particle size distribution of (a) parent emulsion (PE), (b–c) reconstituted emulsion (RE) from powder stored at 20 °C and 33% RH for 0 and 180 days, respectively.

emulsions can be described by the power law model. The consistent coefficient (K) and flow behavior index (n) for a shear rate range of 1–500 s^{-1} were $K = 0.4837$ Pa s^n, $n = 0.78$ for emulsions obtained at 400 bar ($R^2 = 0.9989$) and $K = 0.8516$ Pa s^n, $n = 0.72$ for those at 600 bar ($R^2 = 0.9990$). The viscosity values at shear rate of 100 s^{-1} were 0.164 and 0.217 Pa s for the emulsions prepared at 400 and

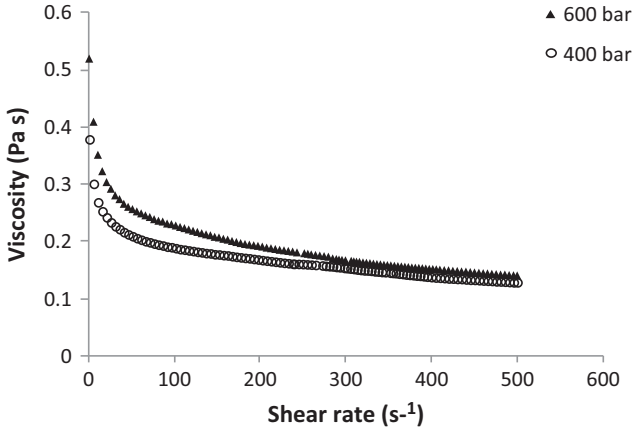

Fig. 2. Effect of homogenization pressure on rheological properties of sodium caseinate (10%) and lactose (10%) stabilized parent emulsions containing 10% of chia seed oil.

600 bar, respectively. This level of shear rate is typical for food processes such as flow through a pipe, stirring or mastication [17].

Emulsions were observed after preparation according to the turbidimetric method. Stable parent emulsions were obtained at both homogenization pressures at least after 600 h of preparation (data not shown).

3.3. Characterization of the microcapsules and reconstituted emulsions

Table 2 lists the physicochemical properties of the microcapsules containing chia oil. Moisture contents and a_w of all spray-dried emulsions ranged from 1.48 to 3.52 g/100 g (d.b.) and from 0.20 to 0.32, respectively, which were affected by the homogenization pressure but not by the inlet/outlet temperature. The main contribution of increasing homogenization pressure may be ascribed to narrow particle size distribution of emulsion droplet size, which can enhance the rate of mass transfer and water evaporation during atomizing as reported by Elversson, Millqvist-Fureby, Alderborn and Elofsson [28]. These results are different from those of Hogan, McNamee, O'Riordan and O'Sullivan [29], who found that moisture contents of spray-dried soya oil emulsions encapsulated with whey protein concentrate (WPC) were not affected by homogenization pressure or oil/protein ratio. The moisture content values were under the minimum moisture specification of dried powder in the food industry (3–4 g/100 g) [30].

The total oil content was similar for the four samples and close to the theoretical value (33.3 g/100 g d.b.). The encapsulated oil content, expressed as microencapsulation efficiency, ranged from 92.1 to 97.4% on total oil (Table 2). These values were higher than those reported by Rodea-González, Cruz-Olivares, Román-Guerrero, Rodríguez-Huezo, Vernon-Carter and Pérez-Alonso [14] for chia seed oil encapsulated with binary biopolymers blend of WPC with gum Arabic (GA) or mesquite gum (MG). These authors achieved the highest encapsulation efficiency (80.7%) from chia O/W emulsions made with 40% total solid content and a core to wall material of 1:3. The differences of microencapsulation efficiency between our results and those reported by these authors [14] may be due to differences in the particle size of the parent emulsions, which were smaller in emulsions formulated with NaCas and lactose (0.33–0.40 μm) than those with WPC/GA and WPC/MG (2.3–3.3 μm). It has been well documented that emulsion droplet size has a pronounced effect on the encapsulation efficiency of different core materials during spray drying [31,32]. These reports show that reducing emulsion size can result in encapsulated powders with higher retention of volatiles and lower content of non-encapsulated oil at the surface of powder particles. Also, the different wall materials used in both works could have affected the microencapsulation efficiency. The choice of the wall material is very important for encapsulation efficiency and microcapsule stability [5]. According to Gharsallaoui, Roudaut,

Table 2
Physicochemical properties of the microcapsules containing chia oil.

Property*	Operative condition			
	400 bar–135/70 °C	400 bar–170/90 °C	600 bar–135/70 °C	600 bar–170/90 °C
Moisture content (%, d.b.)	3.32 ± 0.17[b]	3.52 ± 0.18[b]	1.48 ± 0.44[a]	2.14 ± 0.27[a]
$a_{w25\ °C}$	0.31 ± 0.01[b]	0.32 ± 0.01[b]	0.20 ± 0.02[a]	0.21 ± 0.02[a]
Total oil (%, d.b.)	30.4 ± 0.3[a]	30.7 ± 0.1[a]	29.4 ± 0.1[a]	30.1 ± 0.1[a]
Microencapsulation efficiency (%)	95.0 ± 2.1[a]	92.1 ± 0.7[a]	97.4 ± 1.2[a]	94.5 ± 0.5[a]

* Each value is the mean ± standard deviation of two independent microcapsule manufacturing trials. Means within a row followed by different letters are significantly different ($p \leq 0.05$).

Chambin, Voilley and Saurel [5] the use of some specific compounds can modify the drying properties of microcapsules. The combination of proteins and carbohydrates as encapsulants has been found to be excellent for microencapsulation [33]. Rosenberg and Sheu [34] studied the addition of lactose to a whey protein-based system and found that this sugar appeared to enhance crust formation by improving the drying properties of the wall. The positive effect of lactose has been attributed to the formation of a continuous glass phase of lactose in which the protein chains are dispersed. Thus, the higher microencapsulation efficiency values found in our work could be also due to the inclusion of lactose as wall material.

In addition, there were no significant differences ($p > 0.05$) in microencapsulation efficiency between the four operating conditions studied. These results are similar to those found by Hogan, McNamee, O'Riordan and O'Sullivan [35], who reported that homogenization pressures higher than 100 bar had no effect on microencapsulation efficiency of soybean oil microencapsulated with NaCas. These authors suggest that above that pressure stable emulsions with sufficiently small particle size (<1.45 μm) can be achieved. Regarding air inlet temperature, Klinkesorn, Sophanodora, Chinachoti, Decker and McClements [30] reported similar results to those found in this work. These authors studied the characteristics of microencapsulated tuna oil produced by spray-drying (165, 180 and 195 °C inlet temperature) O/W emulsions containing corn syrup solids and oil droplets surrounded by multilayer interfacial membranes (lecithin:chitosan) and found that the encapsulation efficiency was unaffected by air inlet temperature.

The shape, size, and morphology of the spray-dried microcapsules were studied by SEM. Analysis of the surface topology revealed that all the microcapsules had similar morphology, irrespective of the operating conditions (Fig. 3). The microcapsules were approximately spherical in shape with some small pores and a polydisperse particle size, with a diameter in the range 3–12 μm. According to Polavarapu, Oliver, Ajlouni and Augustin [27] the pores may have arisen from either aeration of the emulsions prior to spray-drying and/or uneven shrinkage of the material during the final drying period.

A relevant functional property of microcapsules in practical applications is their ability to form stable emulsions when redispersed in water. The rate and efficiency of powder dispersion are important in the application of powdered food ingredients [24]. Thus, the laser diffraction technique was used to obtain information about these parameters.

The dispersibility of powdered emulsions was assessed by measuring the change in mean particle diameter and obscuration as a function of time (Fig. 4). The obscuration is sensitive to the total amount of material dispersed in the fluid, while the D [4,3] value is particularly sensitive to the presence of large particles in a sample [24]. When the samples were tested at initial time ($t = 0$), the droplet obscuration increased steeply with agitation time up to ~1.5 min, after which it reached a constant value (~10 and 15% for 600 and 400 bar, respectively) (Fig. 4a). In addition, D [4,3] decreased from 0.60 μm at the beginning to 0.46 μm after 1 min of stirring for powders obtained at 400 bar–135/70 °C, and from 0.66 to 0.53 μm in the case of those made at 400 bar–170/90 °C. Powders produced at 600 bar showed a small decrease of D [4,3] between the beginning and the final of stirring (Fig. 4b). The droplet obscuration and mean particle size remained relatively constant at agitation time longer than 1.5 min. The rapid decrease in particle size and increase in obscuration indicated that the majority of the powder dissolved rather quickly giving a homogeneous suspension [24]. For powders stored at 20 ± 2 °C in 33% RH for 30 days, the droplet obscuration took slightly longer (~2.3 min) to reach a constant value than for the fresh powder. Besides, the large particles took longer to fully disappear in the 30-day-old samples than in the 0-day-old sample (Fig. 4c–d). According to Klinkesorn, Sophanodora, Chinachoti, McClements and Decker [24], this increase in dispersion time for the older samples could have been caused by the collapse of the particle structure during storage. A decrease in the surface area of powder particles exposed to the surrounding water phase is associated with lower rates of dispersion. For samples stored for 180 days, only those obtained at 400 bar reached a constant obscuration value, whereas the particle size D [4,3] decreased steadily in all cases during the 5 min of stirring from 8–15 μm to 2–6 μm. These values are much higher than those reported at $t = 0$ and 30 days, which may be related to the presence of undissolved powder aggregates, indicating that powders did not disperse efficiently into aqueous phase after 180 days of storage.

The mean particle diameter of spray-dried emulsion was measured during storage of the powder at 20 °C and 33% relative humidity (RH) after its reconstitution into water to a concentration of 10 g solid/100 g. The emulsion reconstituted from microcapsules obtained by drying emulsions at 400 bar (135/70 or 170/90 °C) presented a higher initial particle size (D [4,3] = 0.48–0.54 μm; D [3,2] = 0.31–0.33 μm) than those prepared from emulsions at 600 bar (D [4,3] = 0.33–0.35 μm; D [3,2] = 0.24–0.25 μm) (Table 3). For each operating condition, no significant differences ($p > 0.05$) were found between 0 and 30 days of storage in the particle size. However, after 180 days of storage the particle size of the reconstituted emulsion

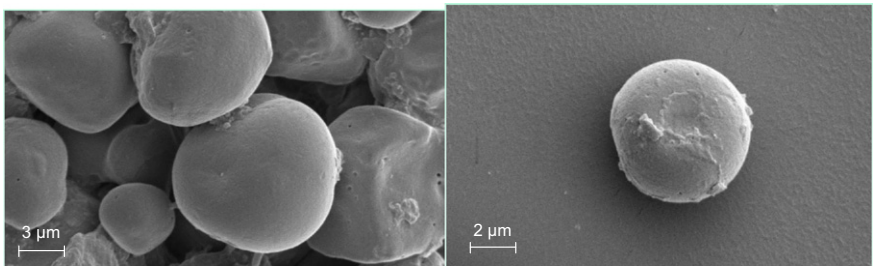

Fig. 3. Micrographs of the outer morphology of chia oil micrcapsules.

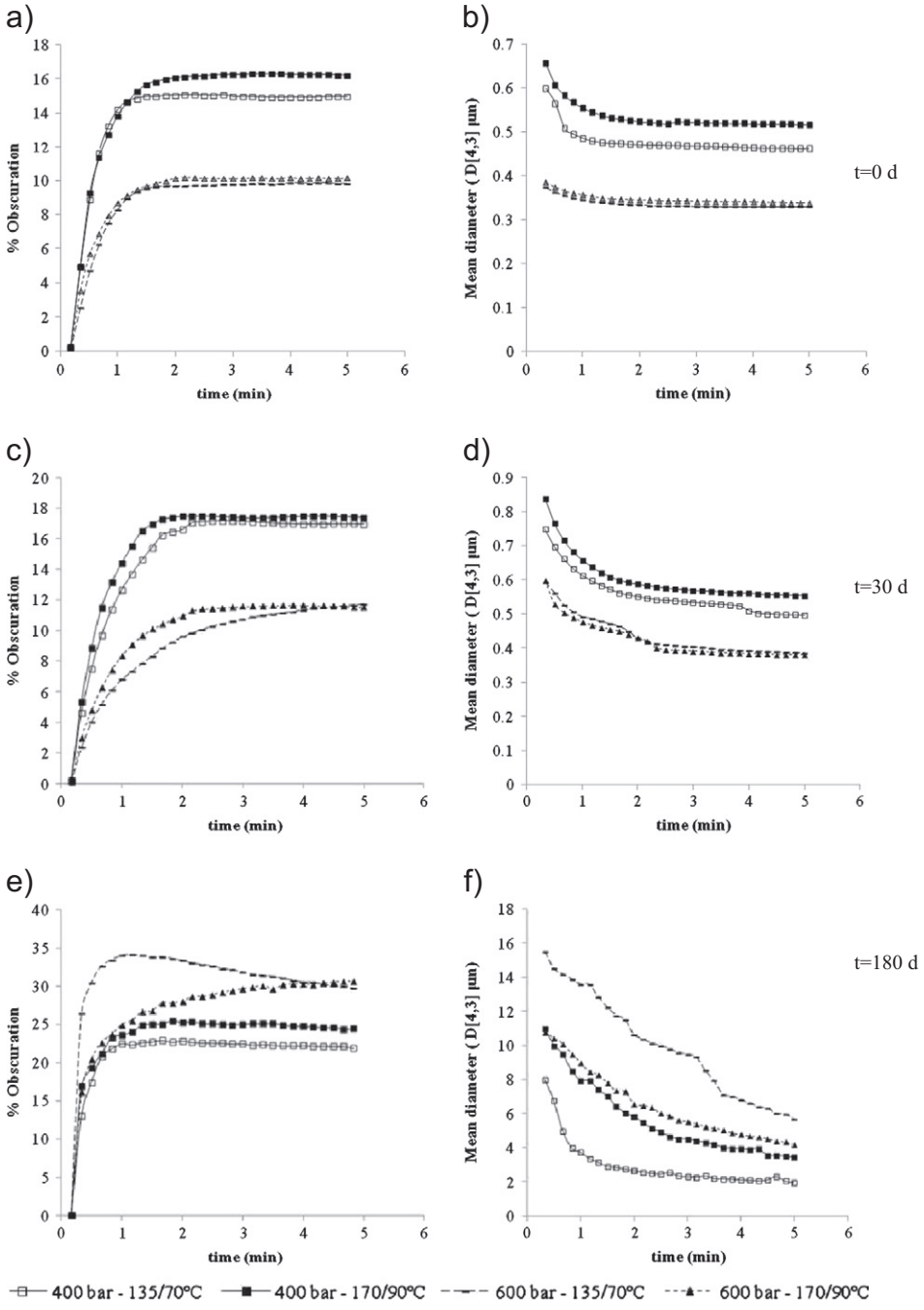

Fig. 4. Influence of stirring time on obscuration and mean diameter of spray-dried chia oil powders stored at 20 °C and 33% RH for 0, 30 and 180 days.

Table 3
Particle size (D [4,3] and D [3,2]) of reconstituted O/W emulsions during storage (0–180 days) at 20 °C and 33% relative humidity.

Operative condition	D [4,3] (μm)			D [3,2] (μm)		
	Storage time (d)			Storage time (d)		
	0	30	180	0	30	180
400 bar–135/70 °C	$0.48 \pm 0.03^{b\,A}$	$0.57 \pm 0.02^{c\,A}$	$1.12 \pm 0.19^{a\,B}$	$0.31 \pm 0.01^{b\,A}$	$0.32 \pm 0.01^{b\,A}$	$0.42 \pm 0.01^{a\,B}$
400 bar–170/90 °C	$0.54 \pm 0.04^{b\,A}$	$0.53 \pm 0.01^{c\,A}$	$3.00 \pm 0.87^{ab\,B}$	$0.33 \pm 0.00^{b\,A}$	$0.33 \pm 0.01^{b\,A}$	$0.98 \pm 0.14^{a\,B}$
600 bar–135/70 °C	$0.33 \pm 0.02^{a\,A}$	$0.46 \pm 0.01^{b\,A}$	$6.02 \pm 2.10^{ab\,B}$	$0.24 \pm 0.00^{a\,A}$	$0.23 \pm 0.00^{a\,A}$	$0.84 \pm 0.23^{a\,B}$
600 bar–170/90 °C	$0.35 \pm 0.04^{a\,A}$	$0.33 \pm 0.01^{a\,A}$	$18.04 \pm 7.47^{b\,B}$	$0.25 \pm 0.01^{a\,A}$	$0.26 \pm 0.01^{a\,A}$	$2.64 \pm 0.60^{b\,B}$

^{ab}Mean values ± standard deviation (n = 2) followed by different small letters in each column differ at $p \leq 0.05$ between operative condition for each storage time, according to Tukey (HSD) test.
^{AB}Mean values ± standard deviation (n = 2) followed by different capital letters in each row differ at $p \leq 0.05$ between storage time for each operative condition, according to Tukey (HSD) test.

(RE) droplets increased significantly for all samples. The particle size distribution of the reconstituted emulsions at the initial time of storage (t = 0 day) (Fig. 1b) was rather similar to those of the corresponding parent emulsions (Fig. 1a). Only a small proportion of droplets greater than 1 μm were observed in the reconstituted emulsion obtained at 600 bar regarding the parent emulsion. After 30 days of powder storage, the particle size distribution of the reconstituted emulsions changed from a unimodal to bi- and multi-modal distribution (Fig. 1c). These changes were more evident in the emulsions reconstituted from powder obtained at 400 bar, showing a considerably wide range of droplet size, which indicated the formation of some larger particles (either flocculated or coalesced droplets).

Physical stability of the different reconstituted O/W emulsions (10% solids) was studied recording the backscattering (BS) profiles as a function of the cell length and time, by a Vertical Scan Analyzer (QuickScan). Fig. 5 shows the profiles obtained for the emulsions reconstituted from the microcapsules prepared at the different operative conditions and stored for 1 week in quiescent conditions. The initial mean values of back scattering along the entire tube (BS_{av0} from BS profile at t = 0) for 400 bar–135/70 °C, 400 bar–170/90 °C, 600 bar–135/70 °C and 600 bar–170/90 °C were 57.07%, 60.43%, 58.42% and 58.15%, respectively. At this time (t = 0), the distribution of particles was homogeneous along the tube. Reconstituted emulsions from 400 bar-microcapsules destabilized by creaming and flocculation, which are more pronounced in the case of microcapsules obtained at 135/70 °C (Fig. 5a, b). The profiles displayed in the reference mode (not shown) had a peak at the bottom of the tube typical of migration of small particles. Then, there was a great increase in BS at the top of the tube which suggested migration of aggregates/flocs. In reconstituted emulsion from microcapsules obtained at 400 bar–135/70 °C (Fig. 5a), there was also a decrease in BS mean values (BS_{av}) indicative of an increment in the mean diameter particle. This behavior may be associated to flocculation [36]. BS is a parameter directly dependent on the particle's mean diameter. BS value always change (moves up or down) with particle size. In general but not always, the BS flux increases with the particle mean diameter when the particles are smaller than the incident wavelength (λ) and it decreases with the mean diameter for particles larger than the incident wavelength [20]. Thus, the aggregates/flocs formed during destabilization of reconstituted emulsions could have a particle size higher than λ (0.8 μm), which cause the decrease of BS_{av} value. The BS profile of the emulsion reconstituted from microcapsules obtained at 600 bar (Fig. 5c, d) showed that little creaming occurred. The top of the profile recorded a shape indicative of the migration of individual particles. However, the clarification degree was still low after 190 h since the serum phase was optically opaque and no light reached the transmission detector (transmission % ~0). Thus, the 600 bar-reconstituted emulsions proved to be the most stable. These results are related to the lower particle size of the reconstituted emulsions at t = 0 when 600 bar was used (Table 3 and Fig. 4b). It is worth noting that a high concentration of small particles produces a slow creaming process, according to the Stokes' law [37].

Fig. 6 shows the evolution of PV for chia bulk oil and spray-dried chia oil powders during storage at 20 °C and 33% RH. The PV values ranged between 2.1 and 2.7 meq/kg oil at t = 0, with no significant differences ($p > 0.05$) between the samples. The relatively low hydroperoxide level in the fresh powder would suggest that chia oil was relatively stable to oxidation during the spray-drying process. These results are similar to those reported by Klinkesorn, Sophanodora, Chinachoti, McClements and Decker [24] in spray-dried tuna oil. Chia bulk oil showed significantly higher PV than microencapsulated oil during storage. The differences among the treatments were observed after the powders were stored for 60 days. At this time, the microcapsules obtained at 600 bar–170/90 °C had the highest PV (11.7 ± 0.8 meq/kg oil) which was significantly different from the other samples. After 100 days of storage, PV of the powders were in the following order: 600 bar–170/90 °C > 600 bar–135/70 °C > 400 bar–170/90 °C ~ 400 bar–135/70 °C; the microcapsules obtained at 400 bar recorded PV <10 meq/kg oil. This PV was

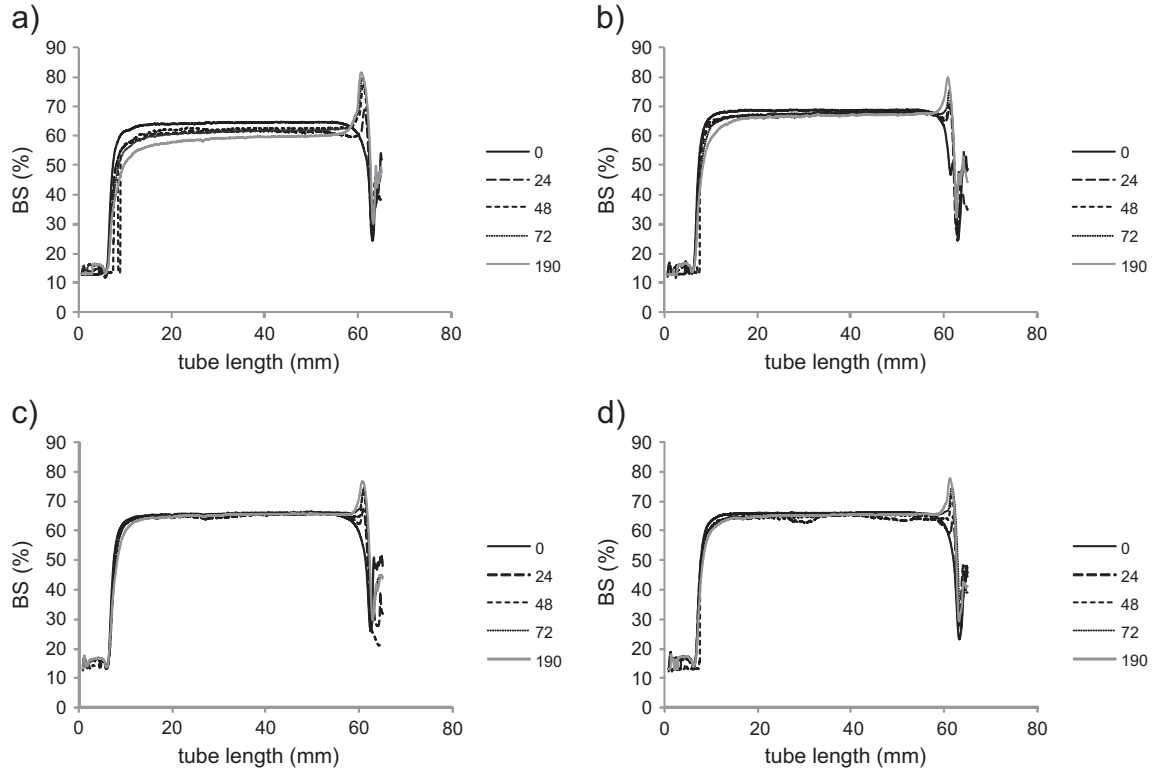

Fig. 5. Backscattering profiles of reconstituted emulsions (10 g solids/100 g) evaluated by the Quick Scan Vertical Scan Analyzer. Data are reported as a function of time (~0.05–190 h) and sample height of emulsion (~0–65 mm). a) 400 bar–135/70 °C; b) 400 bar–170/90 °C; c) 600 bar–135/70 °C; d) 600 bar–170/90 °C.

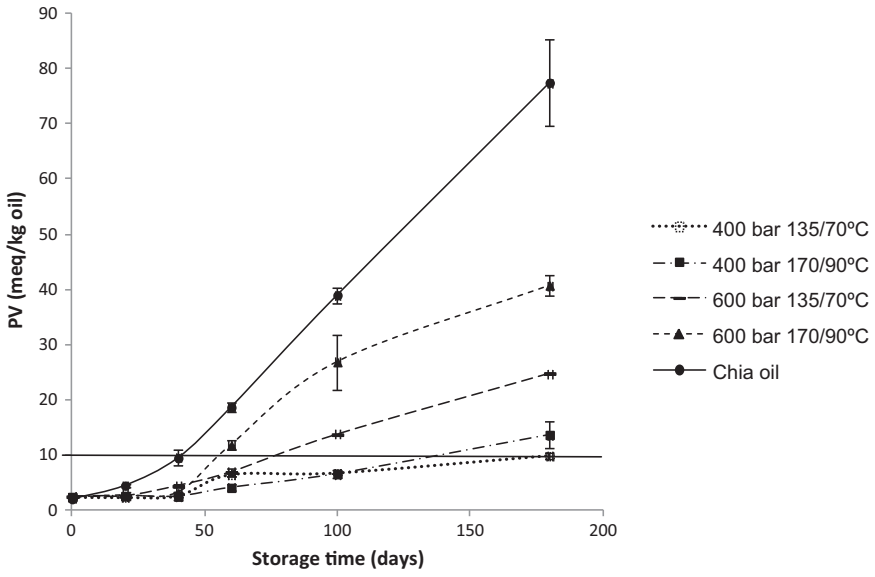

Fig. 6. Peroxide value of chia oil and spray-dried chia oil powders stored at 20 °C and 33% RH during 180 days. Values are the mean ± standard deviation of two independent batches (n = 2).

established by the *Codex Alimentarius* [38] as the acceptable level for human consumption for oils not covered by individual standards and it is also considered the upper limit of acceptability for vegetable oils high in polyunsaturated fatty acids (PUFAs) [39].

Reineccius [40] reported that the oxidation reactions can be induced during spray-drying. At this stage, the oxidation products developed are in the initial stage and might be still in the form of oxidation precursors or free radicals only. This assumption is based on the fact that the particle exposure to heat is in the range of a few seconds at the most. The particle size of the parent emulsion may influence the amount of oxidation precursors formed during the spray-drying process, and thus the subsequent formation of hydroperoxides during storage. Therefore, the smaller particle size of the emulsion at 600 bar produces a higher contact surface with heat than those obtained at 400 bar. In addition, smaller droplets have a larger contact area between prooxidative transition metal ions and lipid hydroperoxides present at the oil–water interface [41,42]. When the drying process has finished, the product is in a solid state and the oxidation reaction rate is slow. However, as storage proceeds, the preformed radicals might progressively change into hydroperoxides, as can be seen by the changes on PV during the storage time studied. In the case of the microcapsules obtained from the emulsions at 600 bar, the inlet temperature effect was also recorded. Increasing the drying air temperature increased the peroxide value. According to Tonon, Grosso and Hubinger [43], the use of higher inlet air temperatures provides more energy available for the lipid oxidation process, which occurs more intensely, favoring the formation of peroxides. These authors observed that the hydroperoxide content of microencapsulated flaxseed oil was higher when the inlet temperature was increased from 138 °C to 202 °C.

4. Conclusions

The results indicate that the microencapsulation process consisting of emulsification using high-pressure valve homogenizer and subsequent spray-drying is suitable for preparing sodium caseinate-lactose-based microcapsules containing chia oil. Results show that the microencapsulation process is efficient (>90%) and allows the achievement of high core retention level. The homogenization pressure for emulsifying had a greater influence on the physicochemical properties of the microcapsules and the reconstituted emulsions than the spray-drying inlet/outlet temperatures. When 600 bar of pressure was used for the emulsification prior to the drying process, the moisture content and a_w of the microcapsules were lower than in the case of 400 bar. Also, the physical stability of the reconstituted emulsions was higher at 600 bar.

Regarding oxidative stability, the microencapsulation has offered protection against oil oxidation during storage. At 400 bar, it was possible to obtain microencapsulated chia oil with PV <10 meq/kg oil after 100 days of storage at 20 ± 2 °C and RH 33%.

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