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Application of Maillard reaction products on chia seed oil microcapsules with different core/wall ratios

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ABSTRACT

This research studies the physical properties of microcapsules formulated with different concentrations of chia oil, using Maillard Reaction Products (MRPs) with different protein:carbohydrate ratio as encapsulants. Microcapsules were obtained from freeze-drying of O/W emulsions composed by non-heated/heated aqueous phases containing NaCas (10%wt) and lactose (10 or 20% wt/wt) blends. Chia oil (10, 15 or 20%wt/wt) constituted the oil phases. The moisture content of microcapsules was 0.31–2.23% d.b., while the water activity was ~0.500. The dispersibility and color were also studied. The microencapsulation efficiency varied between 41.43 and 83.95%. The bulk density was 323–551 kg/m^3 and 244–301 kg/m^3 for tapped and aerated density, respectively. All microcapsules exhibited an outer topography characterized by flakes and agglomerates without cracks or dents. The particle size distribution and D[3,2] of reconstituted emulsions were analyzed. The heat treatment improved the protection of chia oil against lipid oxidation in most samples, partially due to the antioxidant properties of the MRPs. Also, the oil content and the protein:carbohydrate ratio affected de oxidative stability. Thus, MRPs produced by heat treatment of NaCas-lactose mixture with different protein:carbohydrate ratios were effective for conferring microencapsulated chia oil additional oxidative stability.

1. Introduction

Chia (*Salvia hispanica* L., Labiatae) seed contains about 32–38% of oil by weight (Ayerza & Coates, 2005) and is a good source of polyunsaturated fatty acids (PUFAs), mainly ω-3 (α-linolenic ~ 60%), with a low content of saturated fatty acids (SFAs) and bioactive compounds (Ixtaina et al., 2011).

The consumption of ω-3 PUFAs offers multiple health benefits, such as protection against the incidence of coronary diseases, inflammatory disorders, asthma, retina diseases, and helping brain function. Therefore, the incorporation of these compounds in human diet is desirable (O'Dwyer, O' Beirne, Ní Eidhin, O' Kennedyet 2013; Kaushik, Dowling, Barrow, & Adhikari, 2015). Health authorities of different countries have promoted the intake of foods containing high amounts of ω-3 PUFAs, consequently a wide variety of commercial food products enriched with this type of fatty acids has recently been developed (Jacobsen, Sørensen, & Nielsen, 2013).

Chia oil has a high nutritional value associated with its fatty acid profile. However, its high PUFAs content makes it very susceptible to the oxidation process (Ixtaina, Nolasco, & Tomás, 2012). Thus, microencapsulation is a technology that can be used to protect this oil against oxidation during storage and/or processing. Thus, the microcapsules obtained with chia oil could be used as an ingredient to developing ω-3 fortified foods.

The main purposes of this technique are to achieve high microencapsulation efficiency and to provide high oxidative stability of the core. These two objectives are closely related to the process employed for microencapsulation, the composition of the wall material and the core/wall ratio (Gharsallaoui, Roudaut, Chambin, Voilley, & Saurel, 2007; Sanguansri & Augustin, 2007).

Spray-drying and freeze-drying are different processes applied for microencapsulation. Spray-drying is the most widely used process in the food industry since it is economical and flexible. Freeze-drying is a drying process carried out at low temperature, and it could be appropriate for microencapsulation of oils highly sensitive to the oxidation process, such as chia oil. Previous studies

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have shown the benefits of freeze-drying process to obtain microcapsules (Chen, Zhong, Wen, McGillivray, & Quek, 2013; Choi et al., 2007).

The different types of wall materials provide different extents of oxidative stability, depending primarily on their ability to inhibit oxygen transfer (Kaushik et al., 2015). Proteins and carbohydrates are commonly used for microencapsulation of oils with high ω-3 content (Ixtaina, Julio, Wagner, Nolasco, & Tomás, 2015; Sanguansri & Augustin, 2007). The proteins and carbohydrates blends are excellent for microencapsulation (Rosenberg & Sheu, 1996). The emulsification properties of proteins and particularly sodium caseinate (NaCas), seem to offer the functional and physical characteristics necessary to encapsulate lipid core materials (Hogan, McNamee, O'Riordan, & O'Sullivan, 2001). The disaccharide lactose forms a continuous glass phase in which the protein chains are dispersed and improve the drying properties of the wall (Rosenberg & Sheu, 1996). Different researchers have studied the drying of O/W emulsions using NaCas and lactose (Calvo, Hernández, Lozano, & González-Gómez, 2010; Ixtaina et al., 2015; Velasco, Marmesat, Dobarganes, & Márquez-Ruiz, 2006). The Maillard reaction products (MRPs), formed when proteins and carbohydrates with reducing sugar groups are mixed under certain temperature and time conditions, can be used to enhance the oxidative stability of oils with high PUFAs content. The protein-carbohydrate conjugates formed as consequence of Maillard reaction have been shown to have emulsifying and antioxidant capacity. Thus, they have been applied to microencapsulate different oils (Augustin, Sanguansri, & Bode, 2006; Jacobsen et al., 2013; Rusli, Sanguansri, & Augustin, 2006).

Some studies regarding the microencapsulation of chia seed oil have been published (Escalona-García et al., 2016; González, Martínez, Paredes, León, & Ribotta, 2016; Ixtaina et al., 2015; Martínez et al., 2015; Rodea-González et al., 2012). However, none of them reported the use of MRPs as wall material to encapsulate chia oil. This research was carried out to study whether MRPs produced by heat treatment of NaCas-lactose mixture with different protein:carbohydrate and core/wall ratios would be effective for conferring microencapsulated chia oil additional oxidative stability.

The aim of this research was to investigate the effects of the MRPs, the oil concentration and the protein:carbohydrate ratio in the wall on the physicochemical characteristics and oxidative stability of chia seed oil microencapsulated using NaCas and lactose by freeze-drying for the application as functional ingredient in foods.

2. Materials and methods

2.1. Materials

Commercial chia cold-pressed oil was provided by Nutracéutica Sturla S.R.L (Argentina) and stored for 3 days at 4 ± 1 °C without head space protected from light and oxygen.

Sodium caseinate was purchased from Sigma-Aldrich Company (St. Louis, MO, USA), D-lactose monohydrate from Cicarelli Laboratories Reagents S.A. (San Lorenzo, Argentina). All reagents were analytical grade.

2.2. Experimental design

A fully factorial design (3 × 2 × 2), with two replications, was applied to study the effects of three factors, including the MRPs -obtained by heat treatment at 60 °C for 30 min-; the core/wall ratios; and the different concentrations of lactose. Twelve different emulsions were prepared (Table 1) and the microcapsules were produced from them as described in sections 2.3.1 and 2.3.3. The microcapsules were subjected to a storage trial during 30 days. About 15 g of each type of microcapsule was placed in an open Petri dish covered by foil with small holes and placed in desiccators at a relative humidity of 33% (using supersaturated solution of $MgCl_2$) at room temperature.

2.3. Methods

2.3.1. Emulsion preparation

Chia oil-in-water (O/W) emulsions were composed of NaCas (10% in weight (wt)), different lactose concentrations (10 or 20% wt/wt), and 10, 15 or 20% (wt/wt) of chia oil (Table 1).

Prior to emulsification, the NaCas was dissolved in distilled water at 50 °C using magnetic agitation. For emulsions containing lactose without heat treatment, the carbohydrate was incorporated in the aqueous phase at 25 °C. In the case of emulsions with lactose and heat treatment, the protein-carbohydrate mixture was heated at 60 °C in a water-bath and held for 30 min in order to promote the MRPs (Augustin et al., 2006). Nisine (0.0012 g/100 g) and potassium sorbate (0.1 g/100 g) were used to prevent microbial growth.

Preliminary homogenization was performed for 1 min at 9500 rpm using an Ultra Turrax T-25 (IKA Labortechnik, Germany), equipped with a S25N-18G dispersing tool. The resultant pre-emulsions were further subjected to a second stage of homogenization in a Panda 2K high pressure valve homogenizer (GEA Niro Soavi, Parma, Italy) at 600 bar, with four recirculation cycles.

2.3.2. Parent emulsion characterization

2.3.2.1. Particle size distribution and mean diameter. The particle size distribution of the emulsions was determined by light scattering using a Mastersizer 2000 instrument equipped with a Hydro 2000MU as dispersion unit (Malvern Instruments Ltd., Worcestershire, UK) (Ixtaina et al., 2015). The pump speed was settled at 2000 rpm. The refractive index of the disperse phase was 1.47. The droplet size was reported as Sauter diameter (D [3, 2]), which estimates the specific surface area of the emulsions (Ixtaina et al., 2015).

The Span value was calculated according to Eq. (1):

Table 1
Formulations for chia O/W emulsions previous to freeze-drying based on 3 × 2 × 2 full factorial design. Experimental parameters and samples codes.

Chia oil concentration (%wt/wt)	Lactose concentration (% wt/wt)			
	10		20	
	Heat treatment		Heat treatment	
	Without	With	Without	With
10	10OC10L	10OC10LHT	10OC20L	10OC20LHT
15	15OC10L	15OC10LHT	15OC20L	15OC20LHT
20	20OC10L	20OC10LHT	20OC20L	20OC20LHT

$$Span = \frac{(d(v,90)) - (d(v,10))}{(d(v,50))} \quad (1)$$

where $d(v,10)$, $d(v,50)$, and $d(v,90)$ are diameters at 10%, 50%, and 90% cumulative volume calculated from the particle size distribution curves, respectively.

2.3.3. Preparation of microcapsules by freeze-drying

First, the samples were frozen. For that, the emulsions (100 g) were placed into plastic trays (12.5 cm × 16.0 cm), frozen at −20 ± 1 °C for 48 h and then transferred to −80 ± 1 °C for 24 h. Following, microcapsules were obtained from the frozen emulsions by freeze-drying in laboratory scale equipment for 48 h. The samples were ground using a manual mortar and sifted using a plastic mesh equivalent to ASTM No. 7 sieve in order to standardize the powder size.

2.3.4. Microcapsule characterization

2.3.4.1. Moisture content. The moisture content of the chia oil powders (2 g) was measured gravimetrically by drying the microcapsules (24 h, 70 °C, 29 in Hg) in a vacuum oven (Instrumentación Científica S.A., Buenos Aires, Argentina) (Baik et al., 2004).

2.3.4.2. Water activity. This parameter was determined using an AquaLab Water Activity Meter CX2 model Decagon Devices Inc, USA, at 25 ± 0.5 °C.

2.3.4.3. Essential fatty acid content. The 18:2 (ω-6) and 18:3 (ω-3) content was determined by ^{1}H NMR spectroscopy. Approximately 300 mg of each sample was weighed and dissolved in 1.5 mL chloroform-d_1. The mixture was ultrasonicated for 30 min and afterwards was shaken for 2 h. Then the mixture was centrifuged and 2 mL of dimethylsulfoxide-d_6 with tetramethylsilane (TMS) was added to the mixture.

A Bruker Avance III 500 MHz spectrometer (Bruker Biospin, Rheinstetten, Germany) with a BBFOPLUS SmartProbe probe equipped with a Bruker Automatic Sample Changer (B-ACS 120) was used to carry out the NMR measurements at ambient temperature. ^{1}H NMR spectra were recorded using a standard 1D pulse sequence (PS) at a 30° flip angle with 512 scans, 131 k time domain, 24.02 ppm spectral width, receiver gain of 90.5, and 5.45 s acquisition time. The data were recorded automatically by ICON-NMR (Bruker Biospin, Rheinstetten, Germany). All NMR spectra were manually phased, baseline-corrected and integrated by a Topspin 3.2 (Bruker Biospin, Rheinstetten, Germany).

Specific NMR regions were used for quantification: δ 2.75–2.85 ppm (18:3) and δ 2.69–2.75 ppm (18:2).

2.3.4.4. Microencapsulation efficiency of total oil, ω-6 and ω-3 PUFAs. Microencapsulation efficiency of total oil (ME%) was performed according to Augustin, Bhail, Shen, Oiseth, and Sanguansri (2015) with some modifications. About 1 g of microcapsules was placed on filter paper (Whatman N° 4), washed three times with 10 mL of hexane, collected on a flask and then evaporated under a nitrogen stream. The free oil content was determined by weight difference. It was assumed that the total oil was equal to the initial oil since previous study (Ixtaina et al., 2015) showed that all the initial chia oil remained in the microcapsules. Microencapsulation efficiency was calculated according to Eq (2):

$$ME\% = \left(\frac{\text{Total Oil} - \text{Free Oil}}{\text{Total Oil}}\right) \times 100 \quad (2)$$

Microencapsulation efficiency of ω-6 (ME% $_{\omega\text{-}6}$) and ω-3 (ME% $_{\omega\text{-}3}$) PUFAs were calculated by the data from the fatty acid analysis of encapsulated oil and total oil determined by ^{1}H NMR spectroscopy according to Eqs. (3) and (4):

$$ME\%_{\omega-6} = \frac{\omega-6 \text{ of microencapsulated oil}}{\omega-6 \text{ of total oil}} \quad (3)$$

$$ME\%_{\omega-3} = \frac{\omega-3 \text{ of microencapsulated oil}}{\omega-3 \text{ of total oil}} \quad (4)$$

2.3.4.5. Powder bulk density and compressibility. In addition to the ME of microcapsules, other quality control parameters such as bulk density, Carr Index and Hausner Ratio are used to evaluate the powder flRatio are (Fitzpatrick, 2005). The bulk density can be defined as the mass of a powder divided by the volume occupied by it. The bulk density can be classified as aerated and tapped densities. The aerated bulk density (ρ_A) was analyzed by allowing the dispersed powder to settle in a container due to the gravity influence, whereas the tapped bulk density (ρ_T) was obtained by tapping the container holding the powder. These densities were measured according to Holgado, Márquez-Ruiz, Dobarganes, & Velasco, 2013. For this propose a graduate cylinder (100 mL) with 25 g of powder was used, and the respective densities were calculated according to Eqs. (5) and (6).

$$\rho_A = m_0/v_0 \quad (5)$$

where v_0: volume occupied by the powder (m^3), m_0: powder mass (kg).

$$\rho_T = m_0/v_T \quad (6)$$

wher v_T: volume occupied by the powder after tapping (m^3).

From the parameters previously described, the compressibility (C) was calculated according to Eq (7):

$$C = (\rho_T - \rho_A)/\rho_T \quad (7)$$

2.3.4.6. Microstructure. The microcapsule morphology was study by scanning electron microscopy (SEM). The microcapsules were fixed on a sample holder with graphite tape, and then metalized with gold (SPI Supplies) Sputter. The samples were observed using a FEI-Quanta 200 instrument in high vacuum mode operating at 20 Kv.

2.3.4.7. Color. Samples were homogeneously distributed in a glass Petri dish (diameter 95 mm) and the color of the microcapsule surface was measured using a Minolta colorimeter (CR-400, Konica Minolta Sensing Inc., Japan) calibrated with a white standard tile. Color was recorded using the L^* (lightness) a^* (red-green component) and b^* (yellow-blue component) values of samples.

2.3.4.8. Particle size distribution and mean diameter of the reconstituted emulsion. The dispersion of the powder (solid content 10% wt/wt) was made by stirring the microcapsules in water at room temperature for 30 min. The measurements were carried out according to section 2.3.2.1.

2.3.4.9. Dispersibility. Dispersibility of the microcapsules was determined according to Klinkesorn, Sophanodora, Chinachoti, McClements, and Decker (2005). Samples ~0.3 mg of powder/mL

of distilled water were added within the stirring chamber (500 mL) of a laser diffraction instrument (Malvern Mastersizer Model 2000 E, Malvern Instruments, Worcestershire, UK) spinning at 2000 rpm, measuring changes in mean particle diameter (D [3,2]) and obscuration during 5 min.

2.3.4.10. Oxidative stability. An accelerated oxidation test of the bulk oil and the microcapsules was performed in a Rancimat (Metrohm 679, Switzerland) (AOCS Cd 12b-92, 2013) apparatus using 3.0 g of oil or 1.5 g of microcapsules at 98 °C with continuous bubbling of an air stream at 20 L/h. Stability was expressed as induction time (t_i), in hours.

2.3.4.11. Peroxide value. Peroxide value was evaluated spectrophotometrically according to the method of Diaz, Dunn, MCClements, and Decker (2003). Briefly, the emulsions were reconstituted from the powders according to 2.3.4.7 section. The extraction of lipid hydroperoxides was made by mixing 300 μL of the reconstituted emulsion with 1.5 mL of an iso-octane/isopropanol (3:1 v:v) mixture, vortexing 3 times for 10 s each. The phases were separated by centrifuging and the organic phase was used for analysis. The organic phase was added to 2.8 mL of a methano/butanol solution (2:1 v/v) followed by 15 μL of 3.94 M thiocyanate solution and 15 μL of 0.072 M acidic ferrous iron solution. After 20 min in the dark at room temperature, the absorbance was measured at 510 nm. Lipid hydroperoxide concentrations were determined using cumene hydroperoxide standard curve.

2.3.5. Statistical analysis

Multifactorial ANOVA test was used to analyze the main effects of each factor and the interactions between them. Tukey's High Meaningful Difference test was performed ($p \leq 0.05$) for mean multiple comparisons. Statgraphics Centurion software (Version XV. II for Windows, Manugistics Inc., USA) was used for the statistical analysis.

3. Results and discussion

3.1. Parent emulsion characterization

3.1.1. Particle size distribution and mean diameter

It is important to obtain emulsions with high physical stability due to the relatively long time required for freeze drying, during which possible losses of the material to be encapsulated could occur (Chen et al., 2013). In this sense, the particle size distribution and the mean diameter are relevant because these parameters are closely related to the physical stability of the emulsions.

Fig. 1 shows the particle size distribution curves for the parent emulsions prepared with different protein:carbohydrate ratios and oil concentrations. The particle diameters ranged from 0.1 to 10 and 0.1–239 μm for emulsions with and without heat treatment, respectively. The particle size distribution profiles of the parent emulsions were bimodal, except for sample with 15% of chia oil, 20% of lactose without heat treatment which presented unimodal distribution. In the case of emulsions with a similar protein:carbohydrate ratio and the application of heat treatment, the particle size distribution was narrower than the other ones (Fig. 1). It can also be seen that the Span values of emulsions without heat treatment (1.1770–4.0900) were higher than those with thermal treatment (1.0260–2.5685), showing a lower polidispersibility level in these last systems. A similar result was obtained by Zhang et al. (2015), who observed that the emulsion with MRPs showed the smallest particle size and the narrowest size distribution. This behavior can be explained by the excellent emulsifying property of the protein-polysaccharide conjugates (Akhtar & Dickinson, 2007).

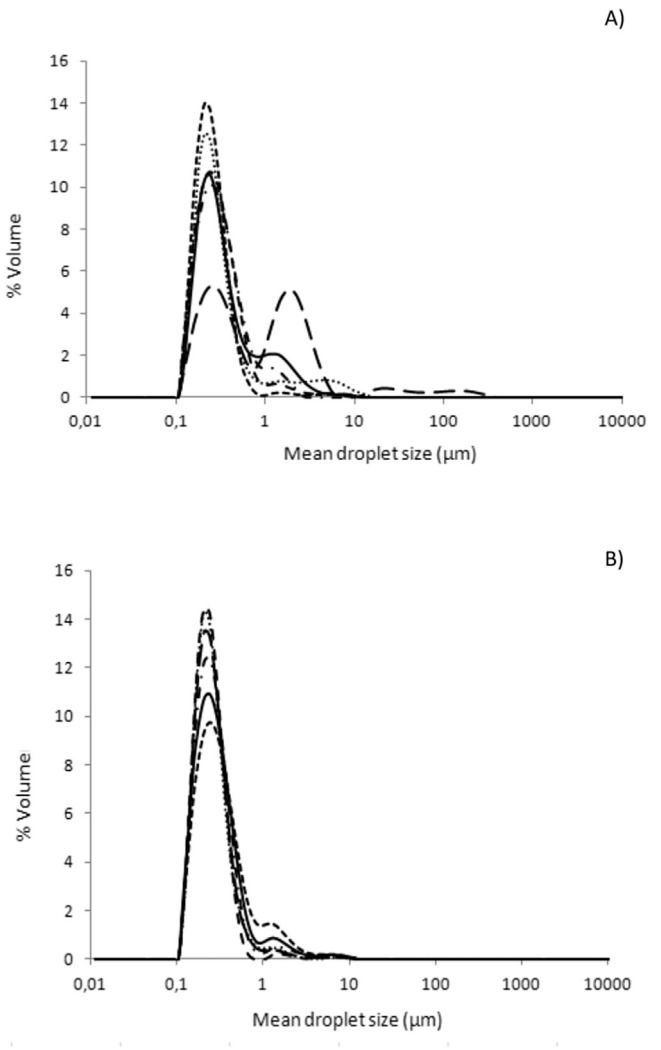

Fig. 1. Particle size distribution (% volume) of parent emulsions: A) without heat treatment B) with heat treatment ▬ · ▬ · 10OC10L; ▬▬ 15OC10L; ▬ ▬ 20OC10L; ▬ ▬ ▬ 10OC20L; ▬▬▬ 15OC20L; ····· 20OC20L.

3.2. Microcapsule characterization

3.2.1. Moisture content (MC)

Table 2 shows that lactose concentration and heat treatment presented a very significant effect ($p \leq 0.001$) on moisture content. Also, double and triple significant interactions were found between factors, except lactose concentration x heat treatment.

The obtained values ranged between 0.31 and 2.23% d.b. (Table 3), which are lower than those required to achieve chia oil microcapsules with a good stability during storage (3–4% d.b.) (Klaypradit & Huang, 2008).

3.2.2. Water activity (a_w)

There were no significant effects ($p > 0.05$) of oil load, lactose concentration or heat treatment on a_w (Tables 3 and 4). All samples

Table 2
Multifactorial analysis of variance (ANOVA) for the physicochemical properties of microcapsules of chia seed oil.

Main effects	D.F.	Sum of squares										
		MC	a_w	ME	ME $_{\omega-6}$	$ME_{\omega-3}$	ρa	ρ_e	C	D[3,2]	Oxidative stability	
											Initial	Final
Oil load (A)	2	0.036	0.000	737.95*	1083.15***	591.53***	1027.0	23172.3	0.011	195.168***	567.496***	331.704
Lactose concentration (B)	1	0.814***	0.002	424.89*	512.24***	499.14***	693.4	13254.0	0.000	30.736**	480.078***	158.569
Heat treatment (C)	1	1.591***	0.000	705.94**	616.61***	623.32***	126.0	2604.2	0.009	58.101***	108.120*	78.156
AxB	2	1.205***	0.000	224.57	165.92***	112.08**	1348.0	13456.0	0.011	65.433**	1618.640***	729.329*
AxC	2	0.700**	0.000	90.12	105.03**	45.23	89.3	25106.3	0.047**	115.943***	242.242**	40.815
BxC	1	0.028	0.000	583.00**	513.84***	702.11***	287.0	5340.2	0.004	13.599	8.378	62.823
AxBxC	2	2.792***	0.002	454.96	347.21***	546.73***	2093.3	11825.3	0.006	28.000*	579.124***	48.160
Pure error	12	0.420	0.007	822.26	68.95	91.77	11583.5	43141.0	0.041	41.574	189.681	1049.950
Total	23	7.588	0.011	4043.70	3413.26	3211.91	17246.6	137899.0	0.130	548.554	3793.760	2499.510

D.F Degree of freedoms; MC moisture content; a_w water activity at 25 °C; ME microencapsulation efficiency of total oil; $ME_{\omega-6}$ microencapsulation efficiency of ω-6 PUFAs; $ME_{\omega-3}$ microencapsulation efficiency of ω-3-PUFAs; ρ_a aerated density; ρ_e packed density; C compressibility index; D[3.2] average oil droplet diameters of the reconstituted emulsions*$p \leq 0.05$. **$p \leq 0.01$; ***$p \leq 0.001$.

Table 3
Physicochemical properties of microcapsules of chia seed oil at initial time (t = 0 d).

Sample	Moisture content (%, d.b.)	$a_{w\ (25°C)}$	Oxidative stability (t_i, h)	Aerated bulk density (kg/m^3)	Tapped bulk density (kg/m^3)	Compressibility Index	Particle size of the reconstituted emulsion D[3,2] (μm)
10OC10L	1.34^{bcd}	0.515^{a}	11.91^{ab}	301^{a}	551^{a}	0.435^{b}	0.283^{a}
15OC10L	0.73^{abc}	0.483^{a}	21.31^{cde}	266^{a}	340^{a}	0.217^{a}	0.450^{ab}
20OC10L	1.02^{abc}	0.495^{a}	15.23^{abc}	244^{a}	323^{a}	0.244^{a}	0.957^{ab}
10OC20L	0.77^{abc}	0.500^{a}	25.13^{de}	266^{a}	450^{a}	0.322^{ab}	0.266^{a}
15OC20L	0.69^{ab}	0.520^{a}	34.87^{f}	270^{a}	402^{a}	0.265^{a}	0.280^{a}
20OC20L	0.31^{a}	0.508^{a}	11.74^{ab}	286^{a}	414^{a}	0.230^{a}	16.778^{c}
10OC10LHT	1.44^{cd}	0.481^{a}	9.62^{a}	260^{a}	351^{a}	0.217^{a}	0.292^{a}
15OC10LHT	2.23^{e}	0.488^{a}	20.29^{bcd}	261^{a}	359^{a}	0.249^{a}	0.390^{a}
20OC10LHT	0.76^{abc}	0.496^{a}	27.73^{def}	255^{a}	352^{a}	0.232^{a}	0.516^{ab}
10OC20LHT	0.74^{abc}	0.522^{a}	51.96^{g}	290^{a}	430^{a}	0.212^{a}	0.292^{a}
15OC20LHT	0.80^{abc}	0.497^{a}	29.33^{ef}	263^{a}	391^{a}	0.264^{a}	0.291^{a}
20OC20LHT	1.99^{de}	0.513^{a}	6.73^{a}	276^{a}	471^{a}	0.306^{a}	2.889^{b}

a_w water activity at 25 °C; t_i, induction time.
Mean values (n = 3). The coefficients of variation were lower than 10%. Different letters in each column indicate differences at $p \leq 0.05$ between formulations, according to Tukey (HSD) test.

Table 4
Microencapsuation efficiency of total oil, ω-6 and ω-3 PUFAs of microcapsules of chia seed oil.

Samples	ME (%)	ME $_{\omega-6}$ (%)	$ME_{\omega-3}$ (%)
10OC10L	83.9^{b}	91.6^{f}	81.4^{f}
15OC10L	74.7^{b}	81.2^{de}	78.4^{ef}
20OC10L	57.4^{ab}	64.3^{b}	58.4^{bc}
10OC20L	73.3^{ab}	79.9^{de}	74.3^{ef}
15OC20L	67.4^{ab}	75.0^{cd}	69.6^{de}
20OC20L	79.7^{b}	82.2^{def}	79.6^{ef}
10OC10LHT	77.2^{b}	86.0^{ef}	77.4^{ef}
15OC10LHT	72.6^{ab}	80.2^{de}	74.0^{def}
20OC10LHT	63.8^{ab}	68.2^{bc}	68.8^{cde}
10OC20LHT	61.7^{ab}	69.1^{bc}	63.1^{bcd}
15OC20LHT	55.4^{ab}	63.5^{b}	56.0^{b}
20OC20LHT	41.4^{a}	46.4^{a}	41.3^{a}

ME% microencapsulation efficiency of total oil; $ME\%_{\omega-6}$ microencapsulation efficiency of ω-6 PUFAs; $ME\%_{\omega-3}$ microencapsulation efficiency of ω-3-PUFAs.
Mean values (n = 3). The coefficients of variation were lower than 10%. Different letters in each column indicate differences at $p \leq 0.05$ between formulations, according to Tukey (HSD) test.

showed values ~0.500, which were lower than 0.6 considered as the upper limit for a food to be microbiologically stable (Fazaeli, Emam-Djomeh, Kalbasi Ashtari, & Omid, 2012; Goyal et al., 2015). These values were higher than those reported by Ixtaina et al. (2015) for the microcapsules obtained by spray drying. Both a_w level and the moisture content of microcapsules obtained in this study would be appropriate for their incorporation in dehydrated food matrices. .

3.2.3. *Microencapsulation efficiency of total oil (ME%), essential fatty acid content and microencapsulation efficiency of ω-6 (ME% $_{\omega-6}$) and ω-3 (ME% $_{\omega-3}$) PUFAs*

The ME% ranged between 41.4 and 83.9% (Table 4), which were lower than those reported by Ixtaina et al. (2015) (~95.0%) for microencapsulation of chia seed oil by spray-drying with NaCas and lactose. A lower ME% for the freeze-drying process in comparison with spray-drying was also found by Chen et al. (2013), who reported that this phenomenon could be produced by the dehydration of emulsifiers during the freezing of water phase, which promotes particle-particle interactions in emulsion and reduces the emulsion stability. Thus, the encapsulated materials could be released from the core when ice crystals are removed during the drying stage.

The statistical analysis showed that the heat treatment was the most relevant factor with a negative impact, reducing the corresponding efficiency of the different microcapsules. A lesser influence was associated with the oil content and lactose concentration (Table 2). In this sense, a negative correlation was found between the total solid content and ME ($r = -0.52$; $p = 0.0090$) and between oil content and ME ($r = -0.43$; $p = 0.0374$). These results show the importance of having sufficient quantities of wall material for encapsulating chia oil. A significant interaction ($p \leq 0.01$) between lactose concentration and heat treatment was found. Thus, for

samples without heat treatment, no significant differences ($p > 0.05$) were detected for ME% between both of the lactose concentrations studied. However, in the case of samples with heat treatment, an increment in lactose concentration caused a decrease in ME%.

The essential fatty acid content of microencapsulated chia oil was 20.6–22.9 g ω-6/100 g of oil and 58.2–65.9 g ω-3/100 g of oil. These values were similar to those of bulk chia oil (ω-6 = 23.4 g/100 g; ω-3 = 60.0 g/100 g), showing that microencapsulation process did not affect the essential fatty acid composition of chia oil. Thus, microcapsules could be used to fortified foods with this type of fatty acids.

Values of ME of ω-6 and ω-3 PUFAs calculated from the ^{1}H NMR spectroscopy data presented a high correlation with those of ME of total oil obtained by the gravimetric analysis ($r = 0.97$; $p = 0.0000$). Thus, the highest ME of total oil, ω-6 and ω-3 PUFAs were found in non-heated samples with 10% of lactose and 10% of oil, while the lowest ones were recorded in heated samples with 20% of oil and 20% of lactose.

3.2.4. Bulk density

Regarding bulk density no effects ($p > 0.05$) of the different factors investigated in the experimental design were recorded (Table 2). Bulk density varied between 323 and 551 kg/m^3 and 244–301 kg/m^3 for tapped and aerated density, respectively (Table 3). These parameters depend on the particle size, distribution and characteristics of the material. Similar values were obtained for Quispe-Condori, Saldaña, and Temelli (2011) for microcapsules with flax oil obtained by freeze drying.

The compressibility in many powders is a measure of internal cohesion, flowability, and to some extent, deformability. A low compressibility indicates a less cohesive powder and a higher bulk density (Onwulata, Konstance, & Holsinger, 1996).

This property did not present significant differences ($p > 0.05$) between the experimental factors studied, but a significant oil load x heat treatment interaction ($p \leq 0.01$) was recorded. This fact is

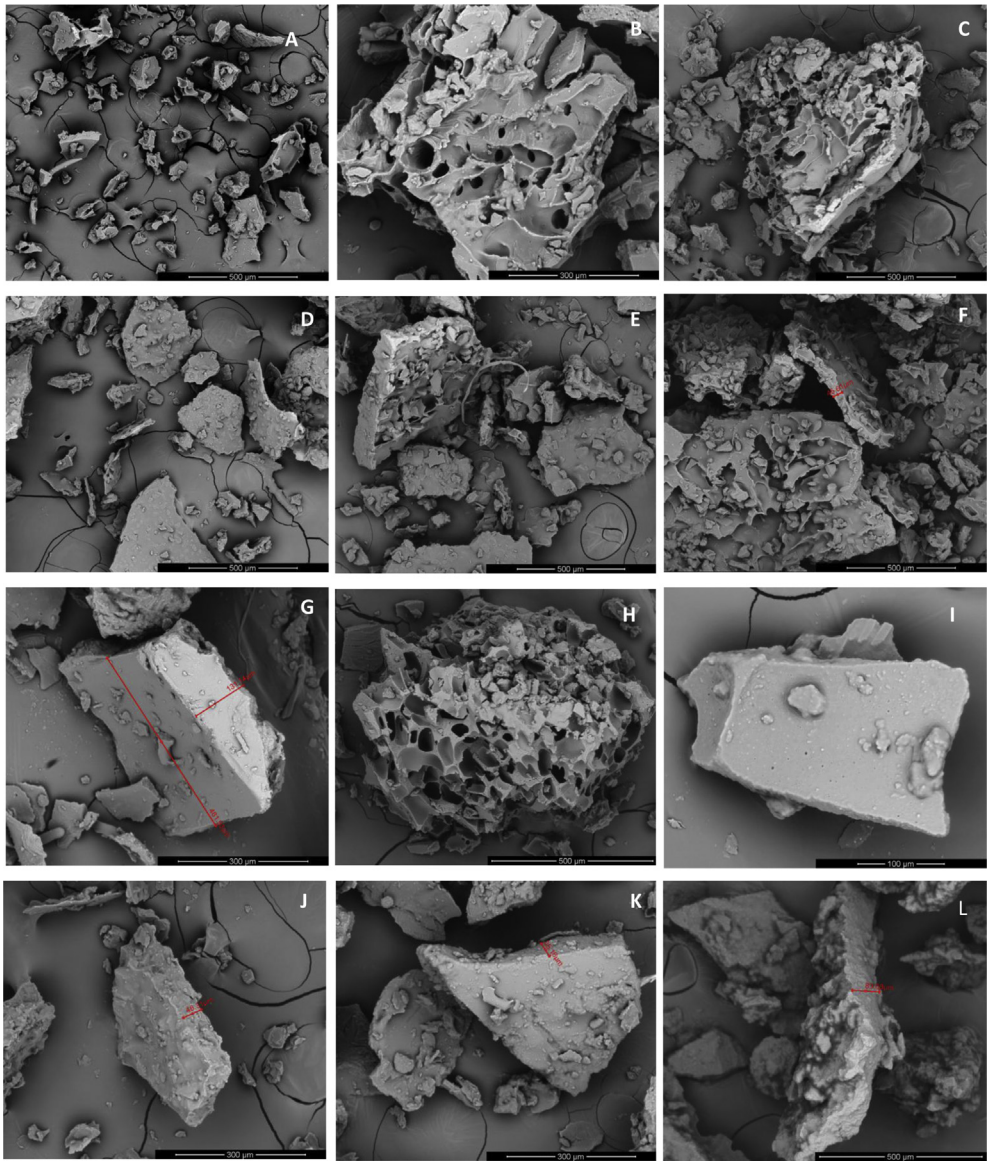

Fig. 2. Micrographs of chia oil microcapsules for different formulations: A) 10OC10L, B) 15OC10L, C) 20OC10L, D) 10OC20L, E) 15OC20L, F) 20OC20L, G) 10OC10LHT, H) 15OC10LHT, I) 20OC10LHT, J) 10OC20LHT, K) 15OC20LHT, L) 20OC20LHT.

Table 5
Color of microcapsules of chia seed oil for different formulations during storage at 20±1 °C.

Samples	t = 0 d			t = 30 d		
	L*	a*	b*	L*	a*	b*
10OC10L	93.51 ± 1.89^{a}	−1.01 ± 0.18^{cd}	12.81 ± 0.89^{a}	91.32 ± 0.78^{a}	−1.37 ± 0.00^{a}	17.84 ± 3.00^{a}
15OC10L	92.21 ± 0.27^{a}	−1.22 ± 0.21^{bc}	14.06 ± 0.16^{a}	94.68 ± 5.27^{a}	−1.71 ± 0.29^{a}	16.79 ± 1.12a
20OC10L	92.89 ± 2.11^{a}	−1.68 ± 0.04^{ab}	14.34 ± 0.34^{a}	90.15 ± 1.78^{a}	−2.22 ± 0.04^{a}	15.59 ± 0.29a
10OC20L	91.94 ± 0.44^{a}	−1.04 ± 0.08^{cd}	13.58 ± 0.28^{a}	89.71 ± 1.77^{a}	−1.66 ± 0.24^{a}	16.38 ± 0.19a
15OC20L	91.49 ± 0.07^{a}	−0.96 ± 0.10^{cd}	14.29 ± 0.34^{a}	90.61 ± 1.05^{a}	−1.58 ± 0.16^{a}	15.87 ± 1.11a
20OC20L	92.70 ± 1.24^{a}	−1.83 ± 0.01^{a}	15.04 ± 0.05^{ab}	89.26 ± 3.37^{a}	−2.41 ± 0.00^{a}	17.92 ± 0.00a
10OC10LHT	91.57 ± 0.38^{a}	−1.01 ± 0.18^{cd}	15.05 ± 1.43^{ab}	88.67 ± 1.34^{a}	−1.50 ± 0.08^{a}	15.48 ± 0.00a
15OC10LHT	93.42 ± 1.58^{a}	−0.98 ± 0.0^{cd}	14.02 ± 1.27^{a}	91.35 ± 0.47^{a}	−1.59 ± 0.24^{a}	19.89 ± 3.75a
20OC10LHT	92.06 ± 0.43^{a}	−1.17 ± 0.17^{bc}	14.775 ± 0.05^{a}	89.85 ± 0.66^{a}	−1.62 ± 0.09^{a}	16.35 ± 0.34a
10OC20LHT	91.47 ± 0.81^{a}	−0.54 ± 0.09^{d}	13.625 ± 0.35^{a}	89.95 ± 0.89^{a}	−0.89 ± 0.14^{a}	15.70 ± 0.65a
15OC20LHT	92.85 ± 1.63^{a}	−0.53 ± 0.03^{d}	13.57 ± 1.00^{a}	91.31 ± 2.55^{a}	−0.85 ± 0.01^{a}	14.54 ± 0.55a
20OC20LHT	90.00 ± 0.38^{a}	−1.27 ± 0.23^{bc}	17.64 ± 0.21^{b}	88.14 ± 2.52^{a}	−1.81 ± 0.03^{a}	18.47 ± 0.20a

Different letters in each column indicate differences at $p \leq 0.05$ between formulations. According to Tukey (HSD) test.

important for the homogeneous character and reproducibility of the microcapsules to be subsequently included in food products (Table 2).

3.2.5. Microstructure

The scanning electronic micrographs (SEM) are shown in Fig. 2. All the formulations exhibited an outer topography characterized by forming flakes and agglomerates with rough appearance without cracks or dents. The pores observed in cases 15OC10L, 15OC10LHT and 20OC10L were possibly formed by the cavities generated by the crystals of ice or bubbles of air retained during freezing. The existence of these pores would not affect the microencapsulation efficiency. Similar results were obtained by Gan, Cheng, and Easa (2008), who worked with microencapsulated fish oil.

SEM micrographs of the microcapsules indicated that as the core:wall ratio increased, the flake size became larger and thicker.

3.2.6. Color

The color of the microcapsules is an important parameter because their incorporation as an ingredient in food products should not significantly alter the characteristics of the product.

The obtained results showed high L^* values (white and luminous), which decreased with storage (except for 15OC10L). Regarding a^* values, this parameters decreased as a function of storage time, whereas b^* values increased. These changes in color parameters showed yellower and a darker appearance at the end of the storage in comparison with the initial microcapsules (Table 5). Binsi et al. (2017) reported that the oxidation of triacylglycerols and free fatty acids can lead to changes in color, indicating the degree of deterioration of foods with high fat content. Thus, the color changes observed during storage would be associated with the oxidation of the surface oil of the microcapsules, which produced colored oxidation products.

3.2.7. Particle size distribution and mean diameter of the reconstituted emulsions

The mean diameter and droplet size distribution of reconstituted emulsions after freeze-drying were analyzed. The reconstitution of emulsions was made with distilled water (1 g solids/10 g emulsion) at ~25 °C for 30 min under stirring (Ixtaina et al., 2015).

Fig. 3 shows the particle size distribution curves of the reconstituted emulsions prepared with different wall protein:carbohydrate ratios and oil concentrations. All reconstituted emulsions showed a bimodal distribution, except the formulation 20OC10L which presented three modes. It was observed that the influence of the heat treatment improved the homogeneity of the systems studied. The same effect of the heat treatment on the width of the distribution had been recorded for the parent emulsions, shown that the good emulsifying property of the protein-carbohydrate conjugates produced from MRP was not affected by the microencapsulation process. In all cases the particle size distribution of the reconstituted emulsions was considerably wider (Span values: 3.1785–31.7865) than those of the parent emulsions. Similar results were reported during the microencapsulation of chia seed oil by spray drying (Ixtaina et al., 2015; Rodea-González et al., 2012). The particle size increased with greater oil and lactose concentration and decreased with the application of heat treatment. This last case

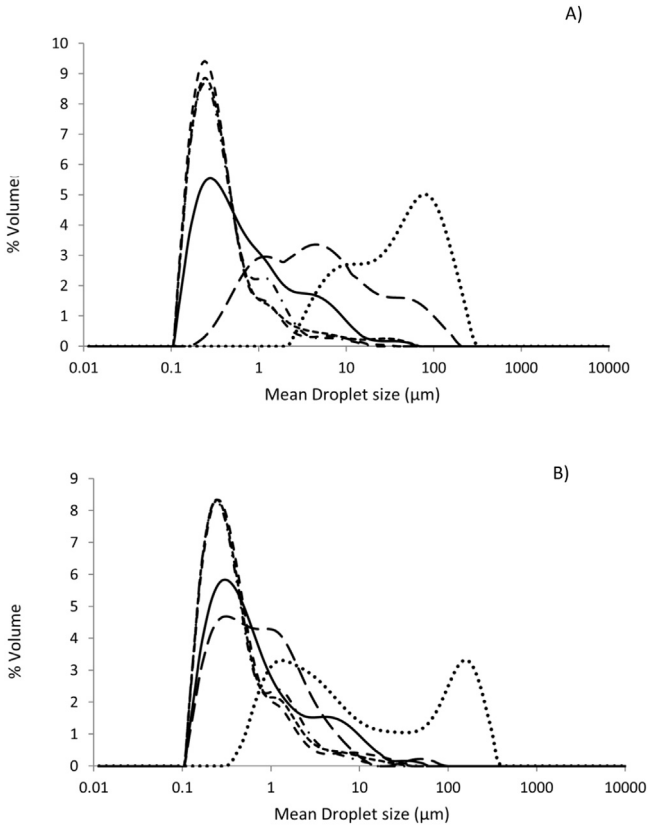

Fig. 3. Particle size distribution (% volume) of reconstituted emulsions: A) without heat treatment B) with heat treatment 10OC10L; 15OC10L; 20OC10L; 10OC20L; 15OC20L; 20OC20L.

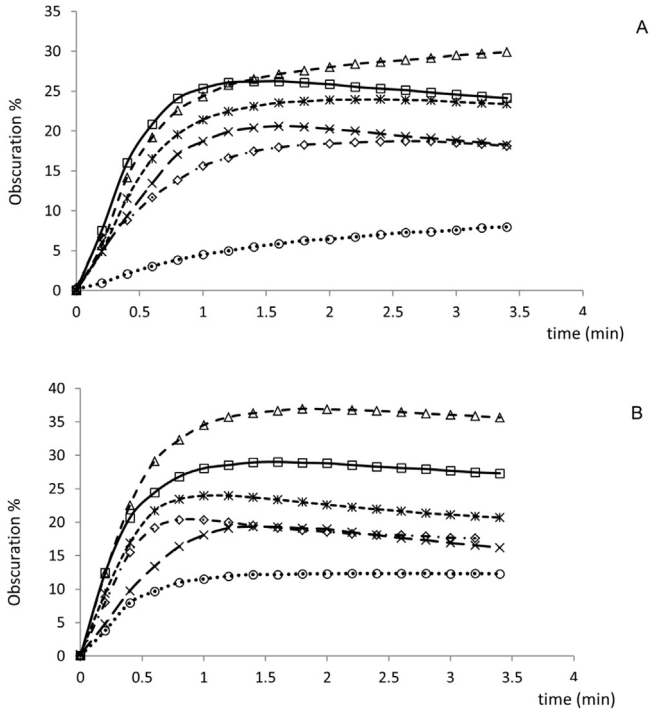

Fig. 4. Influence of stirring time on obscuration of chia seed oil microcapsules: A) without heat treatment B) with heat treatment– ◇– 10OC10L; —□— 15OC10L; – ▲ – 20OC10L; —×— 10OC20L; – –✳– – 15OC20L; ···⊙·· 20OC20L.

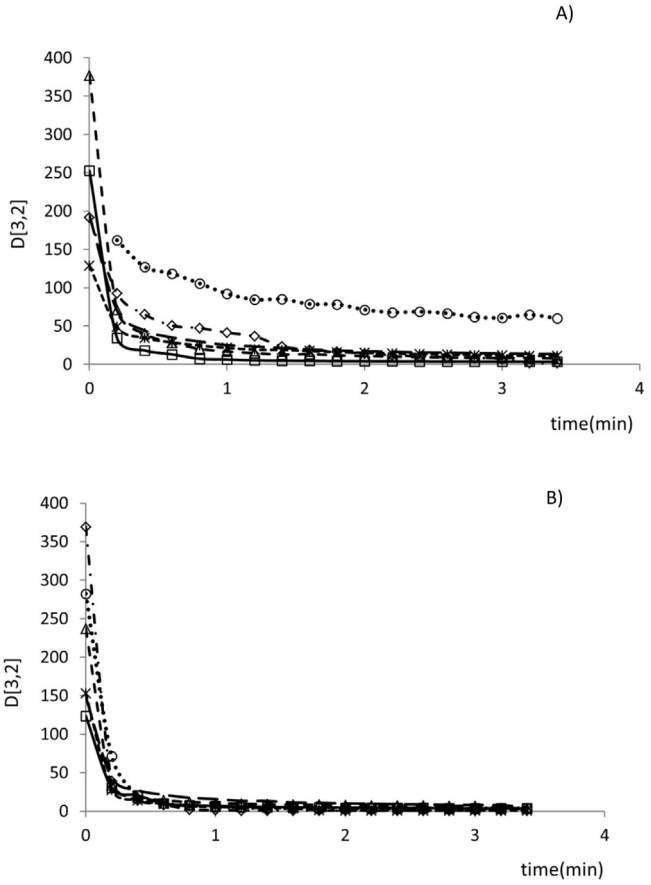

Fig. 5. Influence of stirring time on mean diameter of chia seed oil microcapsules: A) without heat treatment B) with heat treatment.– ◇– 10OC10L; —□— 15OC10L; – ▲ – 20OC10L; — — 10OC20L; – –✳– – 15OC20L; ···⊙·· 20OC20L.

can be explained by the better emulsification obtained, which delays the flocculation. The curves with the highest homogeneity were associated with 10–15% of oil (Fig. 3 A and B). The statistical analysis indicated that the droplet size D[3,2] presented interactions between the factors, being the most important interactions oil load x heat treatment ($p \leq 0.001$) and oil load x lactose concentration ($p \leq 0.01$) (Table 2).

3.2.8. Dispersibility

One of the most important properties of microcapsules is related to the speed and efficiency of powder to disperse in water (Klinkesorn et al., 2005). Therefore, the laser diffraction technique was used to obtain information about this parameter. The dispersibility of powdered emulsions was measured recording the obscuration and mean particle diameter D [3,2] changes as a function of stirring time.

At the initial time of the storage (t = 0), the obscuration increased sharply with the agitation up to approximately 1 min, and then remained constant after that time for most of the samples, except those with 20% of chia oil, 10% and 20% of lactose and not heat treated, which continued to grow slightly (Fig. 4 A). Samples with heat treatment (Fig. 4 B) showed a similar behavior. The highest obscuration values were related to samples with 20% of chia oil and 10% of lactose (with and without heat treatment), whereas those with 20% of chia oil and 20% of lactose recorded the lowest values.

Additionally, the D [3,2] decreased quickly until 0.2 min, after which the particle size remained stable as a function of stirring time (Fig. 5).

The fast reduction in particle size and the increase in obscuration showed that most of the powder dissolved rapidly, giving a homogeneous suspension (Klinkesorn et al., 2005).

These parameters are important because they allow us to evaluate the rehydration of powder. In Fig. 5 it can be seen that the particle size significantly reduced in the first few seconds of stirring, which is very favorable for solubilization and subsequent application in instant foods.

3.2.9. Oxidative stability

The oxidative stability of microencapsulated chia oil was evaluated by Rancimat immediately after drying (t = 0 d) and during storage (t = 30 d). The oxidative stability of the chia oil was effectively enhanced by freeze-drying microencapsulation, since all systems presented higher induction times (t_i) than those corresponding to bulk chia oil ($t_i = 2.46 \pm 0.07$ h). At t = 0 d, the highest induction time was found for the 10OC20LHT sample (Table 3). At this time, the statistical analysis indicated that all factors affected the oxidative stability (Table 2). Also, double and triple interactions between factors were found, except lactose concentration x heat treatment. For samples with 10 and 15% of oil, the highest lactose concentration produced an increase of the induction time. In contrast, samples with 20% of oil showed an inverse behavior. The heat treatment showed a positive effect on the induction time for systems with 20% chia oil, 10% lactose and 10% chia oil and 20% lactose. This can be explained by the formation of protein-lactose conjugates which reduce the amount of hydroperoxides in the powders. It can also be seen that in samples containing 10 or 15% of oil, a 1:2 NaCas:lactose ratio improved the oxidative stability in comparison with those with 1:1ratio. Thus, the protein:carbohydrate ratio is an important factor in the Maillard reaction since a

greater amount of reducing sugars available to participate in the reaction increases its rate and extent. These results suggest that the conjugates obtained by the Maillard reaction in the wall material are appropriated to improve the oxidative stability of microencapsulated chia seed oil. Similar results were obtained by Zhang et al. (2015) in the microencapsulation of fish oil using caseinate and maltodextrin. Research studies have shown that the Maillard reaction antioxidant products are formed as a result of the interaction of sugars with amino acids whether these products are at the interface or in the continuous matrix of the powder (Lingnert, Vallentin, & Erikssonsik, 1979; McGookin & Augustin, 1991). Because this reaction is very common in foods, especially those rich in heat-treated proteins, the heat treatment was applied in this study to promote antioxidant products that could protect the microencapsulated chia oil.

At the end of storage, the induction time decreased significantly for all the samples (data not shown). Only oil load × lactose interaction was found.

A similar trend was recorded for the influence of the heat treatment in terms of PV (data not shown). .

4. Conclusion

Microcapsules of chia oil were investigated in order to evaluate the influence of MRPs, oil concentration and protein:carbohydrate ratio in the wall on the physicochemical characteristic and stability of chia oil. The oil load, the lactose concentration and the heat treatment of the aqueous phase influenced the microencapsulation efficiency of total oil, ω-6 and ω-3 PUFAs, the oxidative stability of microcapsules and the particle size of the reconstituted emulsions. Moisture and water activity levels were low and suitable for dried products. The essential fatty acid composition of microencapsulated chia oil was similar to that of bulk oil, recording high levels of essential fatty acids, mainly ω-3 PUFAs. All formulations exhibited good and fast dispersibility which is important in order to the rehydration properties of powders. The application of the heat treatment was beneficial for most of the variables studied, except for microencapsulation efficiency. The obtained results showed that the MRPs produced by heat treatment of NaCas-lactose mixture with different protein:carbohydrate ratios were effective for conferring microencapsulated chia oil additional oxidative stability.

Conflict of interest

The authors declare no conflict of interest.

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