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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.

Keywords: chia oil; omega-3 fatty acids; microencapsulation; physicochemical properties

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

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 (Sanguansri & Augustin, 2007; Ixtaina, Julio, Wagner, Nolasco, Tomás, 2015). 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, Sørensen, Nielsen, 2013; Rusli, Sanguansri, & Augustin, 2006).

Some studies regarding the microencapsulation of chia seed oil have been published (Rodea-González et al., 2012; Martínez et al., 2015; Ixtaina et al., 2015; Escalona-García et al., 2016; González, Martínez, Paredes, León, Ribotta, 2016).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.

112 2.2. Experimental design

113 A fully factorial design (3x2x2), with two replications, was applied to study the effects
114 of three factors, including the MRPs -obtained by heat treatment at 60ºC for 30 min-;
115 the core/wall ratios; and the different concentrations of lactose. Twelve different
116 emulsions were prepared (**Table 1**) and the microcapsules were produced from them
117 as described in sections 2.3.1 and 2.3.3. The microcapsules were subjected to a
118 storage trial during 30 days. About 15 g of each type of microcapsule was placed in
119 an open Petri dish covered by foil with small holes and placed in desiccators at a
120 relative humidity of 33% (using supersaturated solution of $MgCl_2$) at room
121 temperature.

122

123 2.3. Methods

124 2.3.1. Emulsion preparation

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

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

135 Preliminary homogenization was performed for 1 min at 9,500 rpm using an Ultra
136 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 2,000 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:

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

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 (100g) were placed into plastic trays (12.5 cm × 16.0 cm), frozen at -20±1 °C for 48h 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.

160 2.3.4. Microcapsule characterization

161 2.3.4.1. Moisture content

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

165 2.3.4.2. Water activity

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

168 2.3.4.3. Essential fatty acid content

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

174 A Bruker Avance III 500 MHz spectrometer (Bruker Biospin, Rheinstetten, Germany)
175 with a BBFOPLUS SmartProbe probe equipped with a Bruker Automatic Sample
176 Changer (B-ACS 120) was used to carry out the NMR measurements at ambient
177 temperature. ^{1}H NMR spectra were recorded using a standard 1D pulse sequence
178 (PS) at a 30^{0} flip angle with 512 scans, 131k time domain, 24.02 ppm spectral width,
179 receiver gain of 90.5, and 5.45 s acquisition time. The data were recorded
180 automatically by ICON-NMR (Bruker Biospin, Rheinstetten, Germany). All NMR
181 spectra were manually phased, baseline-corrected and integrated by a Topspin 3.2
182 (Bruker Biospin, Rheinstetten, Germany).

183 Specific NMR regions were used for quantification: δ 2.75-2.85 ppm (18:3) and δ
184 2.69-2.75 ppm (18:2).

185 2.3.4.4. Microencapsulation efficiency of total oil, ω-6 and ω-3 PUFAs.

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

194 $$ME\% = \left(\frac{TotalOil - Free\ Oil}{Total\ Oil} \right) \times 100 \quad (2)$$

195

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

199 $$ME\%_{\omega-6} = \frac{\omega-6 \text{ of microencapsulated oil}}{\omega-6 \text{ of total oil}} \quad (3)$$

200

201 $$ME\%_{\omega-3} = \frac{\omega-3 \text{ of microencapsulated oil}}{\omega-3 \text{ of total oil}} \quad (4)$$

202

203 2.3.4.5. Powder bulk density and compressibility

204 In addition to the ME of microcapsules, other quality control parameters such as bulk
205 density, Carr Index and Hausner Ratio are used to evaluate the powder flRatio are
206 (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)

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

where

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, 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 2,000 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 Díaz, Dunn, MCClements, 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

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

284 **Figure 1** shows the particle size distribution curves for the parent emulsions
285 prepared with different protein:carbohydrate ratios and oil concentrations. The
286 particle diameters ranged from 0.1-10 and 0.1-239 μm for emulsions with and
287 without heat treatment, respectively. The particle size distribution profiles of the
288 parent emulsions were bimodal, except for sample with 15% of chia oil, 20% of
289 lactose without heat treatment which presented unimodal distribution. In the case of
290 emulsions with a similar protein:carbohydrate ratio and the application of heat
291 treatment, the particle size distribution was narrower than the other ones (**Fig. 1)**. It
292 can also be seen that the Span values of emulsions without heat treatment (1.1770-
293 4.0900) were higher than those with thermal treatment (1.0260-2.5685), showing a
294 lower polidispersibility level in these last systems. A similar result was obtained by
295 Zhang et al. (2015), who observed that the emulsion with MRPs showed the smallest
296 particle size and the narrowest size distribution. This behavior can be explained by
297 the excellent emulsifying property of the protein-polysaccharide conjugates (Akhtar &
298 Dickinson, 2007).

299 3.2 Microcapsule characterization

300 3.2.1. Moisture content (MC)

301 **Table 2** shows that lactose concentration and heat treatment presented a very
302 significant effect ($p \leq 0.001$) on moisture content. Also, double and triple significant

303 interactions were found between factors, except lactose concentration x heat
304 treatment.
305 The obtained values ranged between 0.31-2.23 % d.b. (**Table 3**), which are lower
306 than those required to achieve chia oil microcapsules with a good stability during
307 storage (3-4% d.b.) (Klaypradit & Huang, 2008).
308
309 3.2.2. Water activity (a_w)
310 There were no significant effects ($p>0.05$) of oil load, lactose concentration or heat
311 treatment on a_w (Tables 3 and 4). All samples showed values ~0.500, which were
312 lower than 0.6 considered as the upper limit for a food to be microbiologically stable
313 (Fazaeli, Emam-Djomeh, Kalbasi Ashtari, & Omid, 2012; Goyal et al., 2015). These
314 values were higher than those reported by Ixtaina et al. (2015) for the microcapsules
315 obtained by spray drying. Both a_w level and the moisture content of microcapsules
316 obtained in this study would be appropriate for their incorporation in dehydrated food
317 matrices. .
318
319 3.2.3. Microencapsulation efficiency of total oil (ME%), essential fatty acid content
320 and microencapsulation efficiency of ω-6 ($ME\%_{\omega-6}$) and ω-3 ($ME\%_{\omega-3}$) PUFAs
321 The ME% ranged between 41.4 and 83.9 % (**Table 4**), which were lower than those
322 reported by Ixtaina et al. (2015) (~95.0%) for microencapsulation of chia seed oil by
323 spray-drying with NaCas and lactose. A lower ME% for the freeze-drying process in
324 comparison with spray-drying was also found by Chen et al. (2013), who reported
325 that this phenomenon could be produced by the dehydration of emulsifiers during the
326 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 ($p\leq0.01$) 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/100g; ω-3=60.0 g/100g), 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 323-551 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, 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 ($p\leq0.01$) was recorded. This fact is 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 **Figure 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, Easa (2008), who worked with microencapsulated fish oil.

374 SEM micrographs of the microcapsules indicated that as the core:wall ratio
375 increased, the flake size became larger and thicker.
376
377 3.2.6 Color
378 The color of the microcapsules is an important parameter because their
379 incorporation as an ingredient in food products should not significantly alter the
380 characteristics of the product.
381 The obtained results showed high *L** values (white and luminous), which decreased
382 with storage (except for 15OC10L). Regarding *a** values, this parameters decreased
383 as a function of storage time, whereas *b** values increased. These changes in color
384 parameters showed yellower and a darker appearance at the end of the storage in
385 comparison with the initial microcapsules (**Table 5**). Binsi et al (2017)
386 reported that the oxidation of triacylglycerols and free fatty acids can lead to changes
387 in color, indicating the degree of deterioration of foods with high fat content. Thus,
388 the color changes observed during storage would be associated with the oxidation of
389 the surface oil of the microcapsules, which produced colored oxidation products.
390 3.2.7. Particle size distribution and mean diameter of the reconstituted emulsions
391 The mean diameter and droplet size distribution of reconstituted emulsions after
392 freeze-drying were analyzed. The reconstitution of emulsions was made with
393 distilled water (1 g solids/10 g emulsion) at ~25°C for 30 min under stirring (Ixtaina et
394 al., 2015).
395 **Figure 3** shows the particle size distribution curves of the reconstituted emulsions
396 prepared with different wall protein:carbohydrate ratios and oil concentrations. All
397 reconstituted emulsions showed a bimodal distribution, except the formulation
398 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-Gonzalez et al., 2012). The particle size increased with greater oil and lactose concentration and decreased with the application of heat treatment. This last case 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 **Figure 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 ± 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

449 increase of the induction time. In contrast, samples with 20% of oil showed an
450 inverse behavior. The heat treatment showed a positive effect on the induction time
451 for systems with 20% chia oil, 10% lactose and 10% chia oil and 20% lactose. This
452 can be explained by the formation of protein-lactose conjugates which reduce the
453 amount of hydroperoxides in the powders. It can also be seen that in samples
454 containing 10 or 15% of oil, a 1:2 NaCas:lactose ratio improved the oxidative stability
455 in comparison with those with 1:1ratio. Thus, the protein:carbohydrate ratio is an
456 important factor in the Maillard reaction since a greater amount of reducing sugars
457 available to participate in the reaction increases its rate and extent . These results
458 suggest that the conjugates obtained by the Maillard reaction in the wall material are
459 appropriated to improve the oxidative stability of microencapsulated chia seed oil.
460 Similar results were obtained by Zhang et al. (2015) in the microencapsulation of fish
461 oil using caseinate and maltodextrin. Research studies have shown that the Maillard
462 reaction antioxidant products are formed as a result of the interaction of sugars with
463 amino acids whether these products are at the interface or in the continuous matrix
464 of the powder (Lingnert, Vallentin, Erikssonsik, 1979; McGookin & Augustin, 1991).
465 Because this reaction is very common in foods, especially those rich in heat-treated
466 proteins, the heat treatment was applied in this study to promote antioxidant
467 products that could protect the microencapsulated chia oil.
468 At the end of storage, the induction time decreased significantly for all the samples
469 (data not shown). Only oil load x lactose interaction was found.
470 A similar trend was recorded for the influence of the heat treatment in terms of PV
471 (data not shown). .
472
473 **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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Figure captions

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

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

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 - - ✳ - - 15OC20 ···⊙·· 20OC20L

643 **Fig. 5** Influence of stirring time on mean diameter of chia seed oil microcapsules:

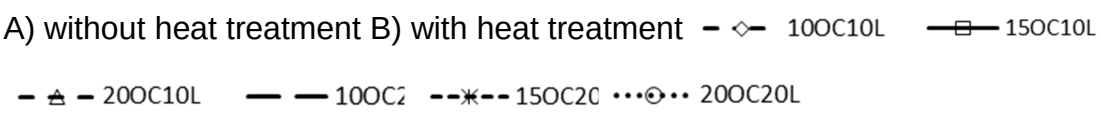
644 A) without heat treatment B) with heat treatment 

Table 1

Formulations for chia O/W emulsions previous to freeze-drying based on 3x2x2 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

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≤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 $_{ω-6}$ (%)	ME$_{ω-3}$ (%)
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%$_{ω-6}$ microencapsulation efficiency of ω-6 PUFAs; ME%$_{ω-3}$ 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≤0.05 between formulations, according to Tukey (HSD) test.

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 ≤0.05. **p≤0.01; *** p≤0.001

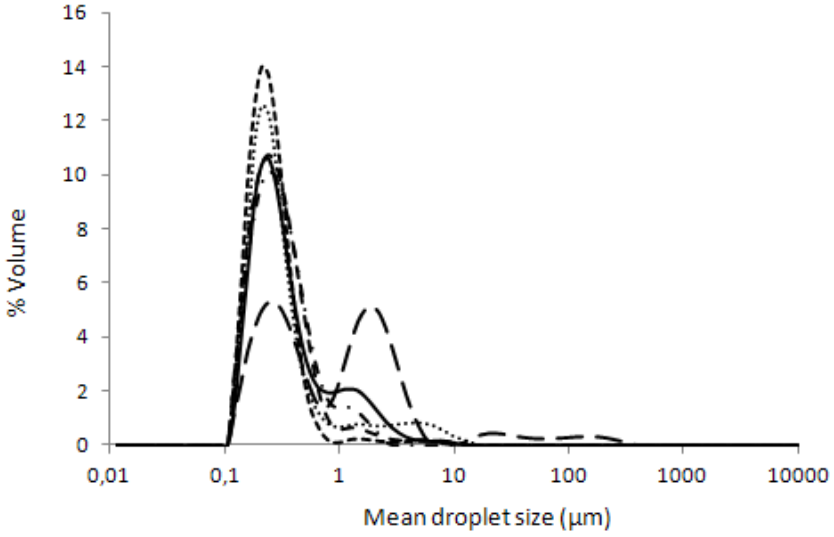
Table 5. Color of microcapsules of chia seed oil for different formulations during storage at 20±1ºC

	t=0 d			t=30 d		
Samples	**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±1.12^{a}
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±0.29^{a}
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±0.19^{a}
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±1.11^{a}
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±0.00^{a}
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±0.00^{a}
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±3.75^{a}
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±0.34^{a}
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±0.65^{a}
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±0.55^{a}
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±0.20^{a}

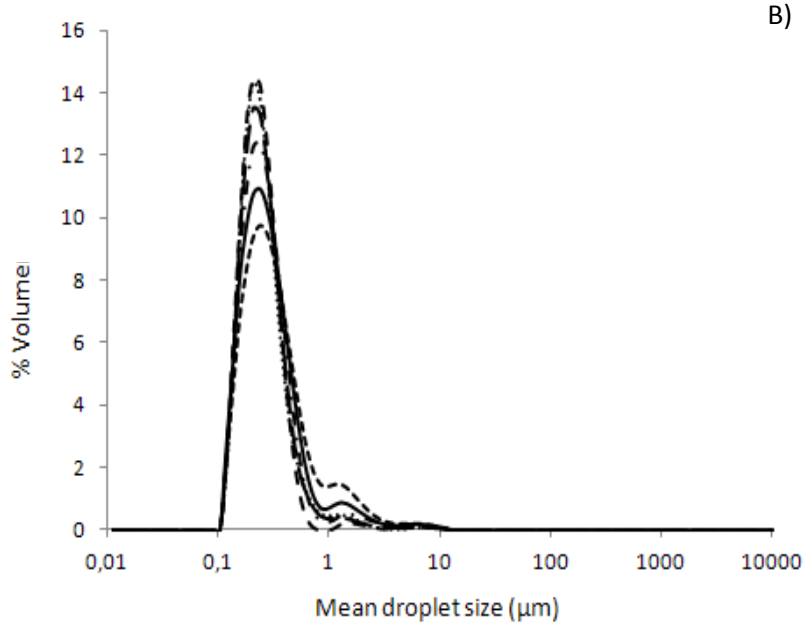
Different letters in each column indicate differences at $p \leq 0.05$ between formulations. according to Tukey (HSD) test

FIGURE 1

A)


16
14
12
10
8
6
4
2
0
% Volume
0,01
0,1
1
10
100
1000
10000
Mean droplet size (µm)

B)


16
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% Volume
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Mean droplet size (µm)

Figure 2.

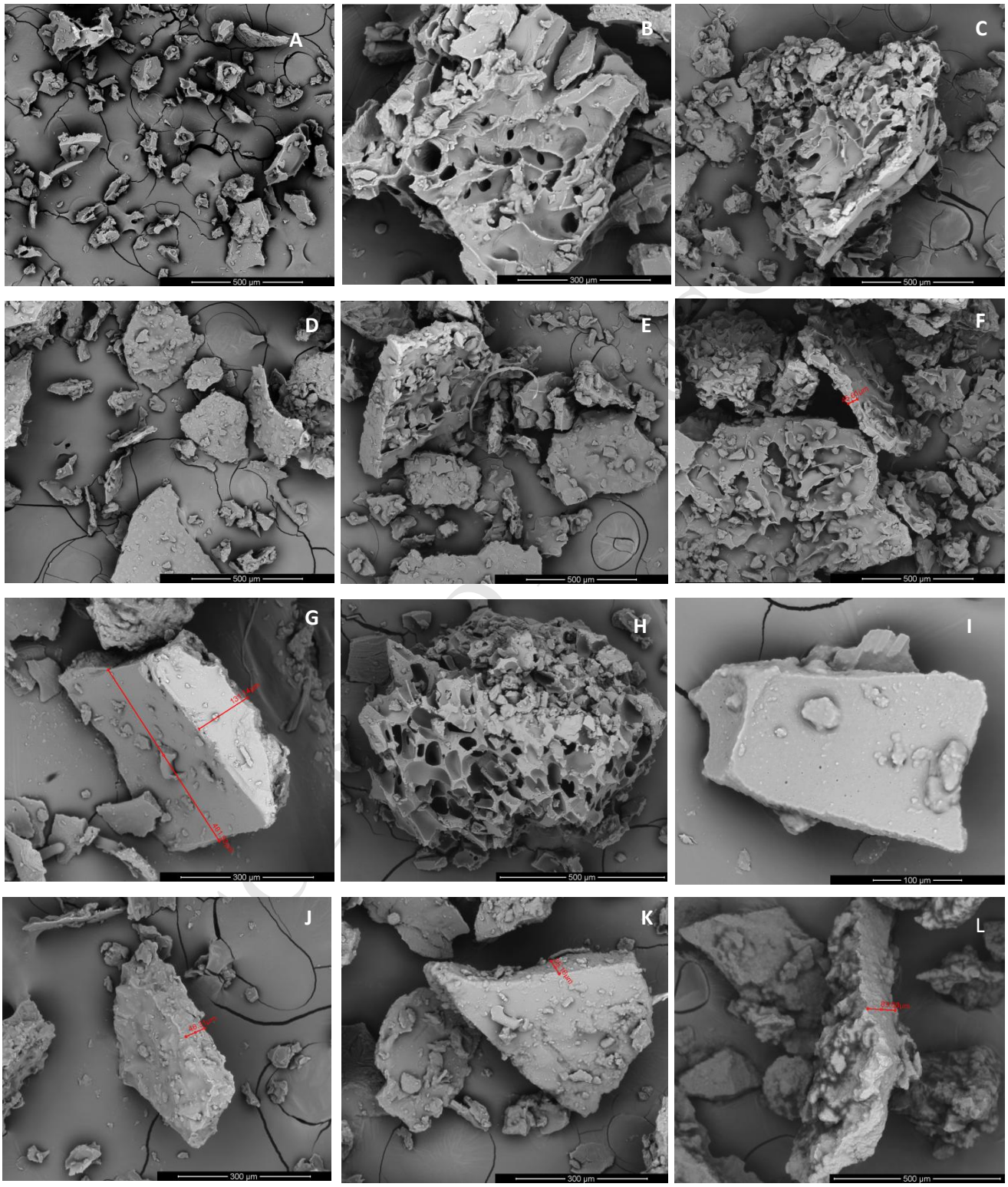

A
500 µm
B
300 µm
C
500 µm
D
500 µm
E
500 µm
F
500 µm
G
300 µm
H
500 µm
I
100 µm
J
300 µm
K
300 µm
L
500 µm

Figure 5

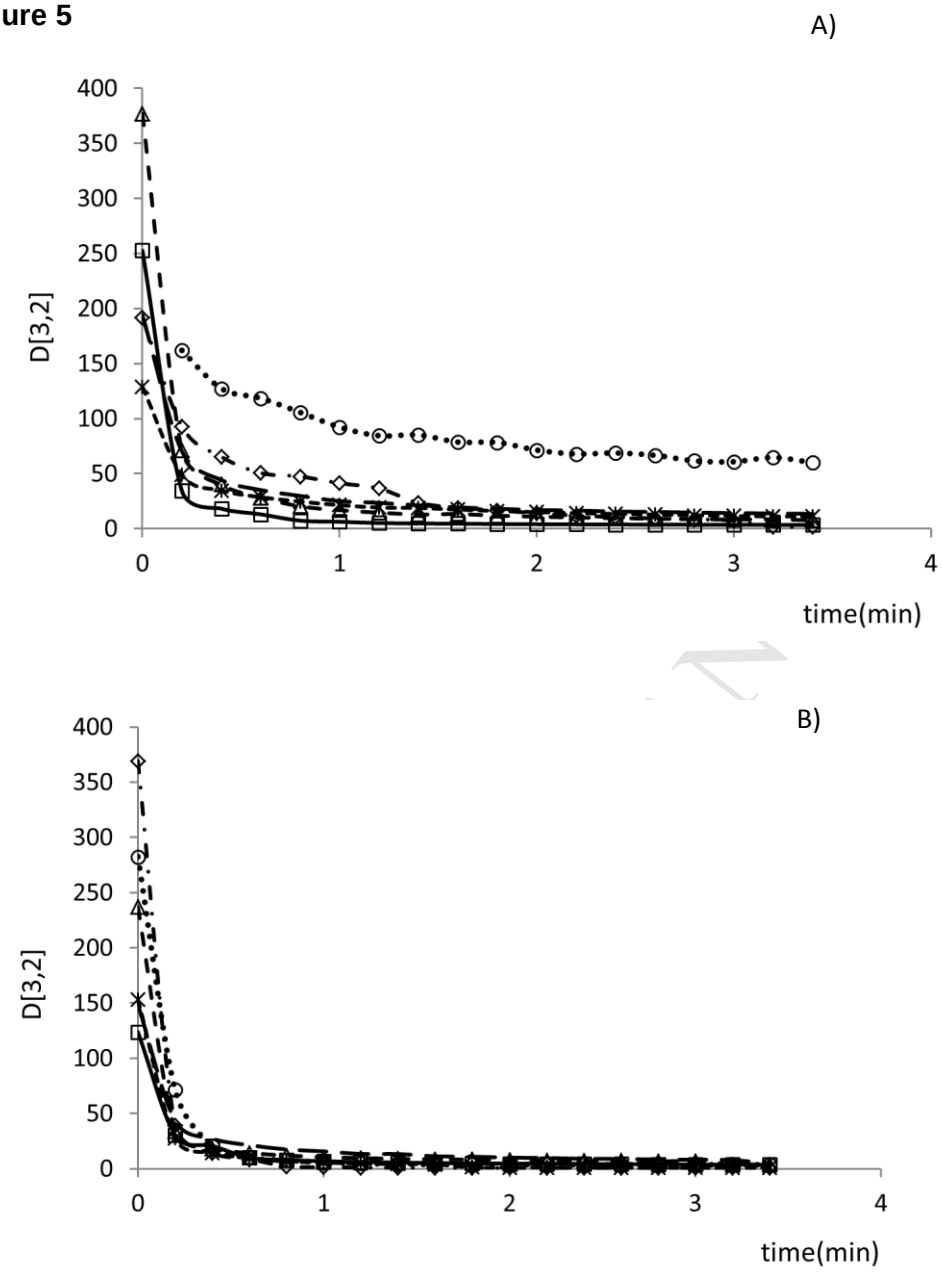

A)
400
350
300
250
200
150
100
50
0
D[3,2]
0
1
2
3
4
time(min)
B)
400
350
300
250
200
150
100
50
0
D[3,2]
0
1
2
3
4
time(min)

Figure 4

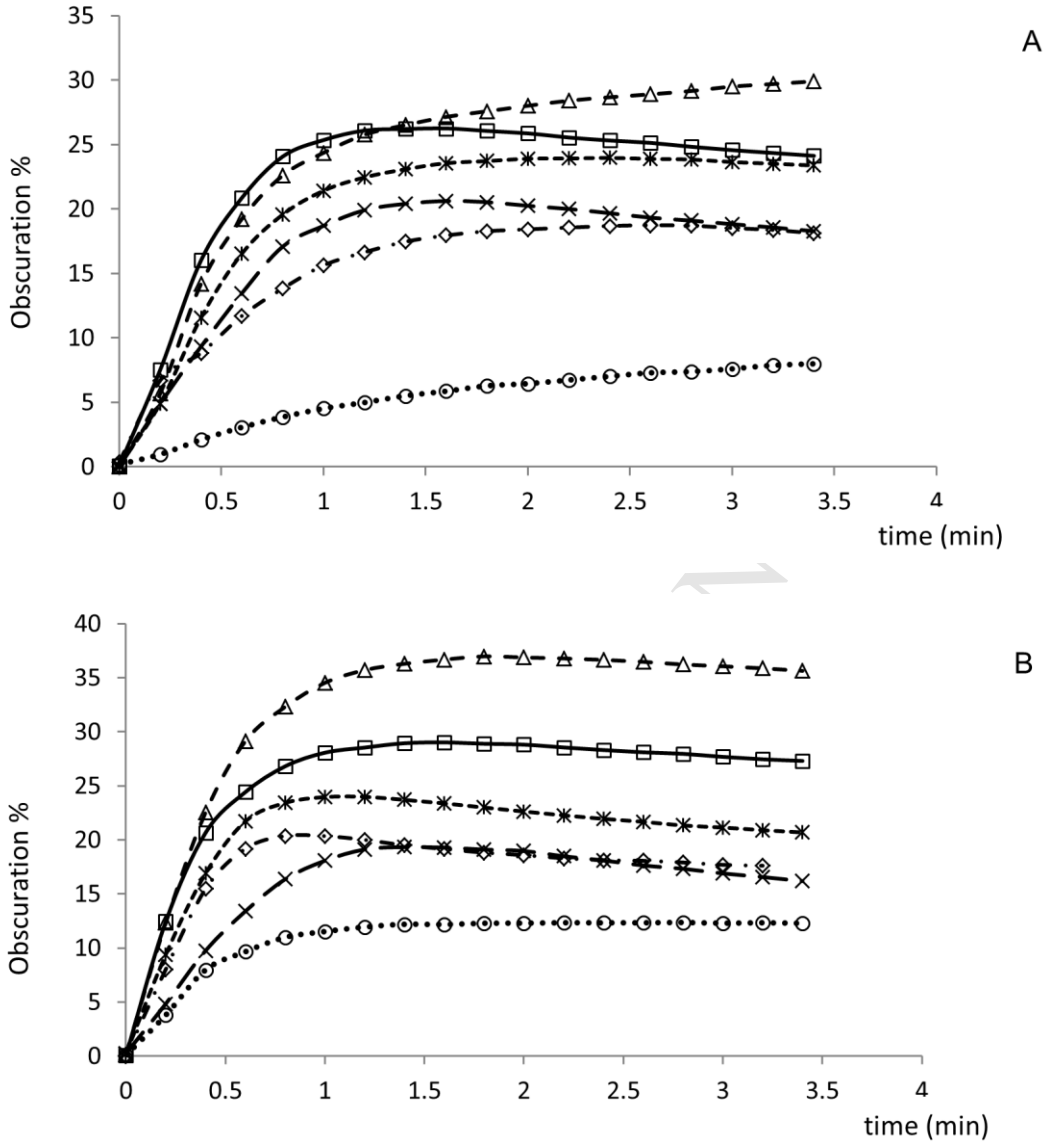
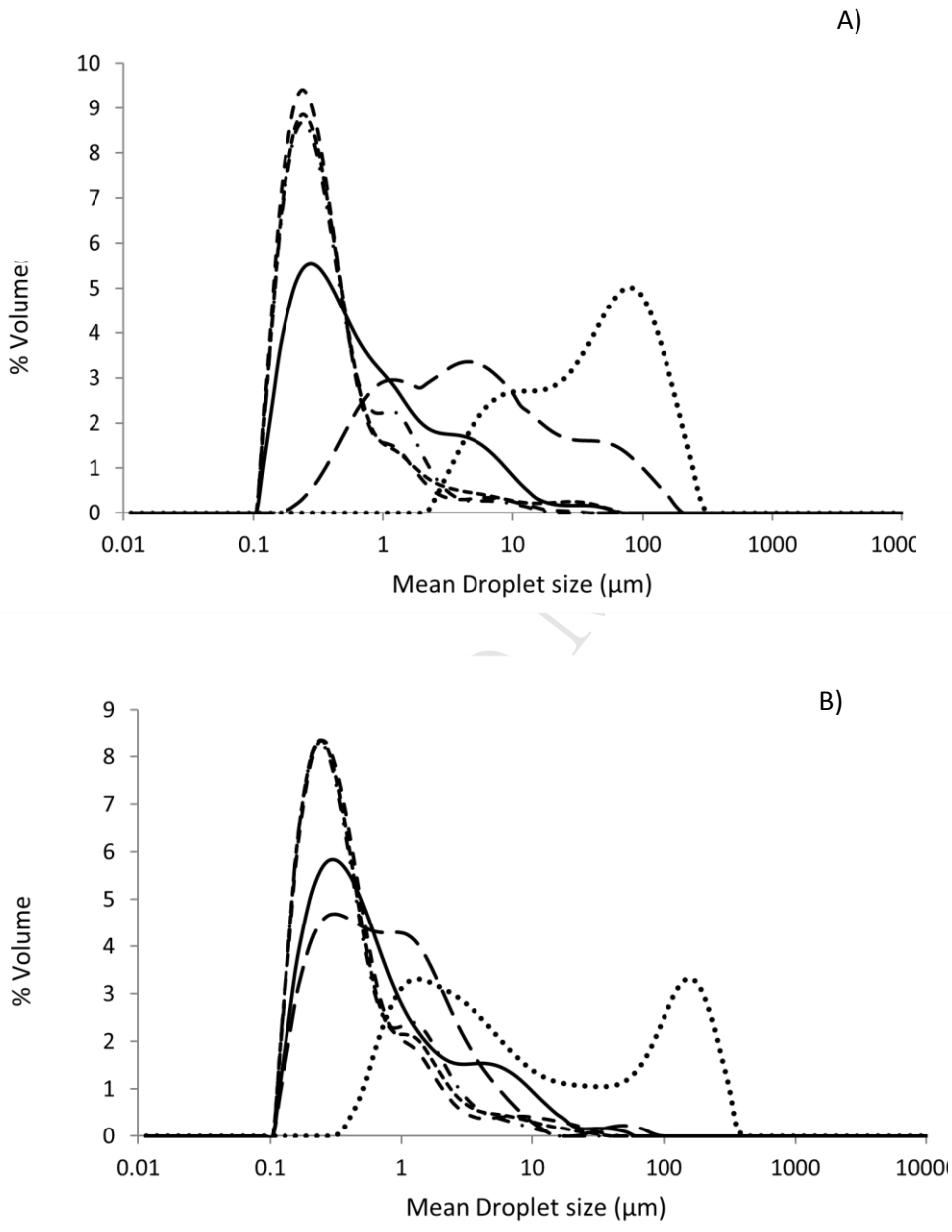


Figure 3



Highlights

* Oxidative protection of chia oil by microencapsulation with diverse core/wall ratio

* Oxidative protection of microencapsulated chia oil by the Maillard Reaction Products

* Highest oxidative stability in systems with 10% oil, 20% lactose and heat treatment

* Highest microencapsulation efficiency in 10% oil-10% lactose-systems (ME> 80%)

* Flake like powder particles with good physicochemical properties and dispersibility