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Chia bilayer emulsions with modified sunflower lecithins and chitosan as delivery systems of omega-3 fatty acids

Luciana M. Julio[a], Claudia N. Copado[a], Bernd W.K. Diehl[b], Vanesa Y. Ixtaina[a,c], Mabel C. Tomás[a,*]

[a] Centro de Investigación y Desarrollo en Criotecnología de Alimentos (CIDCA), CONICET-CIC-Facultad de Ciencias Exactas (FCE) Universidad Nacional de La Plata (UNLP), 47 y 116, La Plata, Buenos Aires 1900, Argentina
[b] Spectral Service AG, Emil-Hoffmann-Straße 33, 50996 Cologne, Germany
[c] Facultad de Ciencias Agrarias y Forestales (UNLP), 60 y 119, La Plata, Buenos Aires 1900, Argentina

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ABSTRACT

Chia oil constitutes a vegetal source of ω-3 polyunsaturated fatty acids (PUFAs) and natural antioxidants, although it is susceptible to the oxidation process. Emulsion-based delivery systems could be adequate for its protection and incorporation into foods. In this study chia bilayer O/W emulsions were obtained applying *layer-by-layer* deposition technique. It consisted of the electrostatic deposition of positively charged chitosan on negatively charged oil droplets. These were stabilized using modified sunflower lecithins (deoiled or phosphatidylcholine-enriched) in presence or absence of maltodextrin. The particle size distribution, mean diameters, ζ-potential and viscosity of emulsions were determined. The chitosan addition had a strong influence ($p \leq 0.001$) on the rheological properties increasing the viscosity and changing the flow behavior of emulsions. The global and oxidative stability of emulsions were monitored during 50 days at 4 °C. The bilayer emulsions showed better physical stability than the monolayer ones. Also, they recorded lower peroxide values ($p \leq 0.05$) than monolayer systems and bulk chia oil, with no significant ($p > 0.05$) changes in their ω-3 PUFAs content during the storage period. Bilayer emulsions with modified sunflower lecithins proved to be protective systems against lipid oxidation, constituting a viable option for the delivery of chia ω-3 PUFAs with potential application in the food industry.

1. Introduction

Omega-3 polyunsaturated fatty acids (ω-3, PUFAs) are associated with numerous potential health benefits when are consumed at adequate levels (Adkins & Kelley, 2010; Lopez-Huertas, 2010). Therefore, big efforts have been made to promote the suitable consumption of them, even more in Western diets characterized by a high ω-6 level and a deficient content in ω-3 fatty acids (Simopoulos, 2002). In this context, chia (*Salvia hispanica* L.) is a vegetable source of these essential fatty acids since α-linolenic acid (~60% ALA) is the predominant FA in its oil. Thus, this oilseed has emerged as a new source of ω-3 PUFAs during the last decades, and its presence in the world market is rapidly increasing. Chia has been considered a food by the US Food and Drug Administration (US Food and Drug Administration, 2009), and the European Parliament and European Council have approved it as a novel food (European Commission, 2009). One of the challenges associated with the development of functional foods including this substrate is related to the adequate preservation of the ω-3 PUFAs, which are very susceptible to lipid oxidation and poorly water-soluble (Augustin & Sanguansri, 2015). To overcome these difficulties, the development of delivery systems like emulsions can provide an efficient protection of these sensitive compounds, and make possible their incorporation into food matrices (Julio et al., 2015).

The emulsification technologies are widely used to obtain food products such as soft drinks, creams, salad dressings, mayonnaise, sauces, soups, butter, and margarine. The emulsions can be used directly or subjected to a drying process to obtain powders (Desai & Jin Park, 2005; Klinkesorn, Sophanodora, Chinachoti, McClements, & Decker, 2005a). In this last case, polysaccharides like maltodextrin are usually added in the formulations as wall materials in microcapsules obtained by dehydration of these emulsions.

Nowadays, the food industry is trying to replace synthetic emulsifiers with natural alternatives (McClements, Bai, & Chung, 2017). Several natural emulsifying agents are used to produce food emulsions including phospholipids, proteins, and polysaccharides. Phospholipids are amphiphilic molecules that can be adsorbed on the oil-water

* Corresponding author. Centro de Investigación y Desarrollo en Criotecnología de Alimentos (CIDCA), Universidad Nacional de La Plata (UNLP), 47 y 116 (1900) La Plata, Argentina.
E-mail address: mabtom@hotmail.com (M.C. Tomás).

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interfaces (Erickson, 2008). Phospholipid-based emulsifiers, commonly referred as lecithins (Klang & Valenta, 2011), may be extracted from numerous sources, such as milk, eggs, rapeseed, cottonseed, soybeans, and sunflower (Bueschelberger, Tirok, Stoffels, & Schoeppe, 2014). Lecithins of vegetable origin -byproducts of the refining process of vegetable oils-are constituted mainly by phosphatidylcholine (PC), phosphatidylinositol (PI), phosphatidylethanolamine (PE) and to a lesser extent by phosphatidic acid and other substances (triglycerides, carbohydrates, etc.). Native lecithin modification process may lead to the production of fractions enriched in a given phospholipid with different composition and functionality. Sunflower lecithin is a promising alternative to the soybean ones because it is from non–genetically modified organism (non-GMO) and its use in the manufacture of foods could be increased by the application of modification processes (deoiling, fractionation, enzymatic modification), resulting in lecithins with different physicochemical and functional properties (Guiotto, Cabezas, Diehl, & Tomás, 2013). Previous studies using high-speed homogenization reported that PC-enriched lecithins produced smaller oil droplets and more stable emulsions than the native ones (Cabezas, Madoery, Diehl, & Tomás, 2012; Guiotto et al., 2013).

Unlike most natural polysaccharides of acidic character employed by the food industry (cellulose, dextrin, pectin, alginic acid, agar, agarose, carrageenans), chitosan is a cationic polysaccharide in acidic environments due to its pK_a of 6.2–7 and the protonation of its amino residues. Chitosan is a natural, non-toxic copolymer of glucosamine and *N*-acetylglucosamine prepared from deacetylation of chitin, which is the major component of the shells of crustaceans. It is found commercially in the waste products of the marine food processing industry (Khanafari, Marandi, & Sanatei, 2008; Limam, Selmi, Sadok, & El Abed, 2011).

Conventional oil-in-water (O/W) emulsions are usually the first systems considered to deliver bioactive lipids because of their relative ease of preparation and low cost (McClements, 2005). In this sense, previous studies have developed conventional emulsion-based delivery systems with chia seed oil using sodium caseinate and chia by-products (Julio et al., 2015, 2016). However, they may exhibit limited physical stability when exposed to heating, cooling, freezing, drying, extreme pH or high minerals concentration and they can be prone to undergo lipid oxidation (Benichou, Aserin, & Garti, 2002; Drusch, 2007). Thus, the development of multilayer emulsions using the *layer-by-layer* (LBL) electrostatic deposition represents an interfacial engineering technology to protect them against lipid oxidation and physical destabilization (Klinkesorn, Sophanodora, Chinachoti, McClements, & Decker, 2005b). This methodology consists in the electrostatic deposition of oppositely charged biopolymers on the oil droplet surface to produce an interface of multiple layers of emulsifying agents and polyelectrolytes (Guzey, Kim, & McClements, 2004).

Different studies have applied the *layer-by-layer* deposition technique in the protection of emulsions to enhance their physical stability (Aoki, Decker, & McClements, 2005; Ogawa, Decker, & McClements, 2003; Perrechil & Cunha, 2013) or to protect different oils rich in ω-3 fatty acids (Fioramonti, Martinez, Pilosof, Rubiolo, & Santiago, 2015; Klinkesorn et al., 2005a). However, none of them have investigated multilayer emulsions with chia seed oil. Additionally, lecithin modifications may be useful for evaluating the potential applications of sunflower by-products to the production of new natural emulsifiers. Thus, the electrostatic deposition of chitosan on different modified sunflower lecithin-coated chia oil droplets would enhance the long-term stability of O/W emulsions, as well as the chemical stability of chia seed oil.

The objective of the present investigation was to study the physicochemical stability of chia O/W emulsions stabilized with differently modified sunflower lecithins through the electrostatic deposition *layer-by-layer* (LBL) of chitosan and the addition of maltodextrin.

2. Materials and methods

2.1. Materials

Chia seed oil, obtained by cold pressing, was provided by Nutraceutica Sturla S.R.L. (Argentina). Fatty acid composition was analyzed by GC according to IUPAC 2.302 standard method (IUPAC, 1992) and it resulted in $C_{16:0}$ 10.5%, $C_{18:0}$ 2.5%, $C_{18:1}$ 6.1%, $C_{18:2}$ 19.5%, $C_{18:3}$ 61.3%. Powdered chitosan (Ch) (medium molecular weight, ~250 kDa, 75–85% deacetylation) was purchased from Sigma Chemical Company (San Luis, MO) and maltodextrin (M) (DE 13–17%) from S.A. Corn Products (Argentina). Native sunflower lecithin was provided by Vicentin S.A.I.C. (Argentina). All reagents were analytical grade.

2.2. Lecithin modifications

Approximately 30 g of native sunflower lecithin were used for the fractionation process with absolute ethanol (3:1 ethanol: lecithin ratio) (Cabezas et al., 2012). For obtaining the ethanol soluble phase, this mixture was incubated at 65 °C for 60 min, centrifuged (1880 g, 10 °C, 10 min) and the solvent removed by evaporation under vacuum.

Ethanol-soluble phase and native lecithin were deoiled with acetone according to American Oil Chemists' Society Official Method Ja 4–46 (AOCS, 1994) to obtain the phosphatidylcholine-enriched (PCL) and deoiled (DSL) lecithins, respectively. Both, the fractionation and deoiling process were performed in duplicate.

2.3. Phospholipid composition

^{31}P NMR spectroscopy was used to determine the phospholipid composition of PCL and DSL using a Bruker Avance spectrometer (Bruker Avance 600 MHz automatic spectrometer, UK) and triphenyl phosphate as internal standard according to Diehl (2008).

2.4. Emulsions preparation

2.4.1. Preparation of stock solutions

Lecithin stock solutions were obtained from the dispersion of 2.2 % wt/wt of DSL or PCL in a stock buffer solution (2 mM sodium acetate and 98 mM acetic acid in water, pH 3.0), separately. These dispersions were stirred and sonicated (1 min, 20 kHz, 70% amplitude) using an ultrasonic processor (Sonics and Materials, Inc., USA) to ensure complete dispersion. Simultaneously, a stock biopolymer solution was prepared by dissolving 1.5 % wt/wt of chitosan in the stock buffer solution and stirring for about 12 h. The final solutions were kept overnight to ensure complete dispersion and dissolution.

2.4.2. Preparation of O/W emulsions

O/W emulsions were obtained by homogenization of 10 % wt/wt of chia oil and 90 % wt/wt of DSL or PCL stock solutions in an Ultraturrax T-25 (IKA- Labortechnik, GmbH & Co., Staufen, Germany) and particle size reduction in a high-pressure valve homogenizer (Panda 2K, GEA, NiroSoavi, Parma, Italy). These concentrated emulsions were diluted with buffer solution to obtain primary O/W emulsions L (5% chia seed oil, 1% DSL) and PC (5% chia seed oil, 1% PCL) and with the addition of maltodextrin L + M (5% chia seed oil, 1% DSL, 20% M) and PC + M (5% chia seed oil, 1% PCL, 20% M) (Fig. 1).

The secondary O/W emulsions were obtained by dilution of the concentrated emulsions with the stock chitosan solution to obtain LQ (5% chia seed oil, 1% DSL, 0.2% Ch) and PCQ (5% chia seed oil, 1% PCL, 0.2% Ch), and with the addition of maltodextrin LQ + M (5% chia seed oil, 1% DSL, 0.2% Ch, 20% M) and PCQ + M (5% chia seed oil, 1% PCL, 0.2% Ch, 20% M) (Fig. 1). In both cases, maltodextrin was dissolved in the buffer solution before their incorporation into emulsions.

Food-grade preservatives, 0.0012% wt/wt of nisine and 0.1% wt/wt

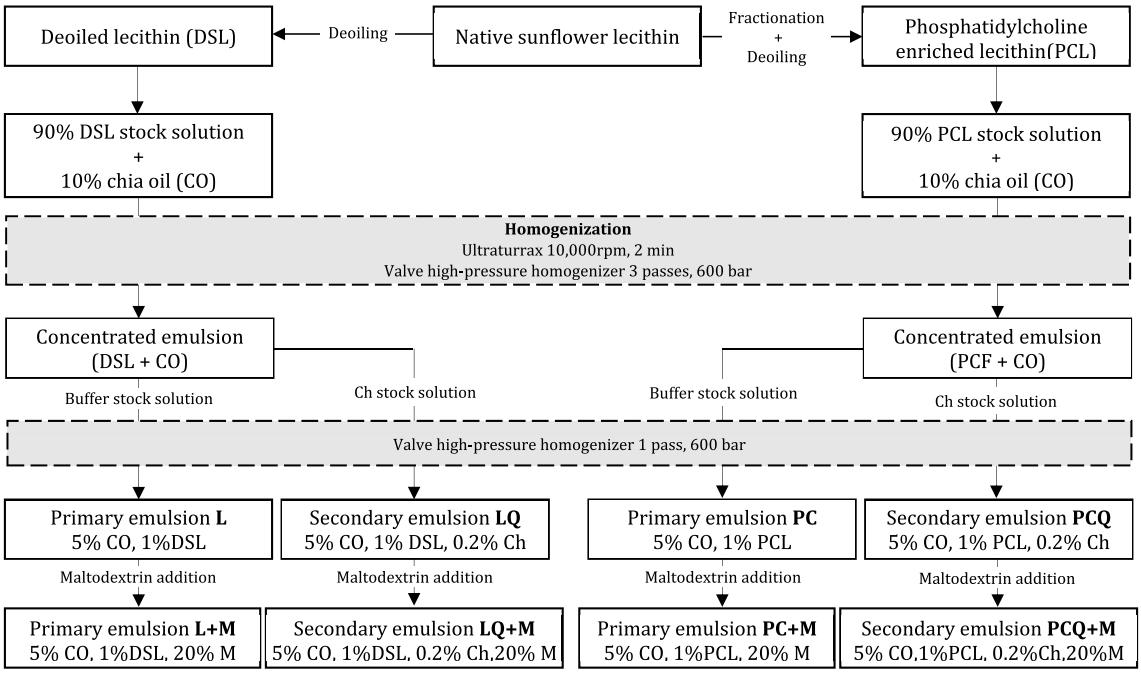

Fig. 1. Flow diagram of chia mono and bilayer O/W emulsions preparation.

of potassium sorbate, were added to the emulsions. All emulsions were stored under dark at 4 ± 1 °C for 50 days.

2.5. Characterization of O/W emulsions

2.5.1. Particle size

The particle size of emulsions was determined using the static light scattering technique with a Malvern Mastersizer 2000E instrument (Malvern Mastersizer 2000E, Malvern Instruments Ltd., Worcestershire, UK). Each sample was suspended directly in a dispersion system (Hydro 2000MU) with buffer solution pH 3.0 at 2000 rpm reaching an obscuration of ~18%. The refractive indices used were 1.47 and 1.33 which correspond to the dispersed and the continuous phase, respectively. Three measurements were performed for each sample. Results were reported as droplet size distribution, mean diameters and Span.

De Sauter ($D_{3.2}$) *surface*-weighted mean diameter (Eq. (1)) was used primarily in the particle size analysis since it is very sensitive to the presence of the small droplets, as they have greater specific surface area (Jafari, Beheshti, & Assadpour, 2013),

$$D_{3.2} = \frac{\sum n_i d_i^3}{\sum n_i d_i^2} \quad (1)$$

where n_i is the number of droplets of diameter d_i.

De Broucker ($D_{4.3}$) *volume*-weighted mean diameter (Eq. (2)) was specifically used only to monitor the changes in the droplet size distribution due to it is more sensitive than D_{32} to analyze the destabilization process (Moran-Valero, Pizones Ruiz-Henestrosa, & Pilosof, 2017),

$$D_{4.3} = \frac{\sum n_i d_i^4}{\sum n_i d_i^3} \quad (2)$$

where n_i is the number of droplets of diameter d_i

Additionally, to determine the distribution width of droplet sizes, the dispersion index (Span) was calculated from Eq. (3),

$$Span = \frac{(D_{0.9} - D_{0.1})}{D_{0.5}} \quad (3)$$

where 90, 10 and 50% of the oil volume in the emulsions is contained in droplets with diameters below or equal to $D_{0.9}$, $D_{0.1}$ and $D_{0.5}$ respectively.

2.5.2. ζ-potential

The electrical charge on the surface of the oil droplets was obtained at 25 ± 0.3 °C by measuring the electrophoretic mobility (μ) using a Zeta Potential Analyzer (Brookhaven 90Plus/Bi-MAS, USA) (Julio et al., 2015). The electrophoretic mobility (μ) was converted using the Smoluchowski equation to ζ-potential values. The measurements were performed in quintuplicate.

2.5.3. Rheological properties

The rheological measurements were determined at 25 ± 0.3 °C using an oscillatory controlled-effort rheometer (Haake RS6000, Thermo Electron Corporation, Germany) equipped with a cylindrical sensor system (Z34 DIN, 34 mm in diameter). The apparent viscosity (η) was recorded by increasing the shear rate ($\dot{\gamma}$) from of 1–500 s^{-1} for 120 s, holding it at 500 s^{-1} for 60 s, and decreasing it from 500 to 1 s^{-1} for 120 s. The determinations were performed in triplicate. The values of η at 100 s^{-1} were reported.

2.6. Emulsion stability

The global stability of emulsions was evaluated using a Vertical Scan Analyzer (Quick Scan, Coulter Corp., Miami, FL) according to Pan, Tomás, and Añón (2002). The emulsions were transferred to cylindrical glass tubes and scanned from the bottom to the top (~65 mm) with a near infrared light source (λ = 850 nm) acquiring the light backscattered (%) through the sample. The sample scans were repeated periodically for 50 days.

2.7. Lipid oxidation of O/W emulsions

2.7.1. Peroxide value (PV)

The primary lipid oxidation products were measured using the PV method. The PV was monitored by the spectrophotometric Shantha and Decker's method as a function of time (Shantha & Decker, 1994). Lipid hydroperoxides were extracted from the emulsion (0.3 mL) with an isooctane/2-propanol mixture (3:1, v/v) (1.5 mL) and subsequent centrifugation 3400*g*, 2 min. Approximately ~0.2 mL of the organic

phase was added to 2.8 mL of methanol/butanol (2:1, v/v) and 30 μL of a thiocyanate/ferrous solution (prepared by mixing 1:1 v/v 0.144 M $FeSO_4$ and 0.132 M $BaCl_2$, centrifuging 3 min and then, mixing the clear ferrous solution with 3.94 M NH_4SCN 1:1 v/v). After 20 min the absorbance of solutions was measured at 510 nm. The lipid hydroperoxides concentration was determined using a standard calibration curve prepared with cumene hydroperoxide.

2.7.2. Proton nuclear magnetic resonance (^{1}H NMR) spectroscopy

The ω-3 fatty acid content of chia seed oil in O/W emulsions were monitored during storage using ^{1}H NMR spectroscopy (Spectral Service GmbH, Cologne, Germany). For the standard sample preparation, 100 mg of each sample was dissolved in 1 mL the mixture $CDCl_3$/MeOD/Cs-EDTA-D_2O 0.2 M (1:1:1) (pH 7.5). The mixture was ultrasonicated for 30 min, shaken for at least 1 h and centrifuged at 4000 rpm. The organic layer was then analyzed. The measurements were carried out on an NMR spectrometer Avance III 500 MHz (Bruker, Karlsruhe, Germany), magnetic flux density 11.7 with Tesla BFOPLUS SmartProbe (5 mm PABBO BB). The number of scans was 16, the spectral width 10 ppm, measurement temperature 298 K, and calibration was performed on the tetramethylsilane (TMS) signal. Processing was accomplished using the software Bruker TopSpin 3.2 Standard operation procedure SAA-GMR045-03, which also used for automatic integral calculation. ^{1}H NMR determinations were carried out in triplicate.

2.7.3. Thiobarbituric acid-reactive substances (TBARs)

The TBARs were determined by Hu and Zhong (2010) method with some modifications. Previous to the test, the TBA solution was prepared by dissolving 15 g of trichloroacetic acid (TCA), 0.75 g of 2-thiobarbituric acid (TBA) and 0.8 g of 2,6-di-*tert*-butyl-4-methylphenol (BHT) in 100 mL of a ternary solvent mixture (1: butanol, isopropanol and 0.5 M HCl 2: 2: 1 v/v/v). For the assay 150 mg of each emulsion was mixed with TBA solution to a total volume of 5 mL. The tubes were incubated at 95 °C for 2 h and then immediately cooled and centrifuged. Finally, the absorbance of the samples at 532 nm was measured. The standard curve using 1,1,3,3-tetraethoxypropane (TEP) was obtained. Results are presented as mmol malonaldehyde equivalent/kg oil.

2.8. Experimental design and statistical analysis

A 3 × 2 fully factorial design (n = 2) was used to study the effect of sunflower modified lecithin type (deoiled and phosphatidylcholine-enriched lecithin), chitosan (0 and 0.2 % wt/wt) and maltodextrin addition (0 and 20 % wt/wt) on each variable. The variables studied were droplet size distribution, mean diameter, ζ-potential, rheological properties, backscattering profiles, primary and secondary oxidation products.

The experimental results were analyzed by multifactorial ANOVA ($p \leq 0.05$) to study the main effect and the interactions between them, using the Statgraphics Centurion XV.II program for Windows software (StatPoint Technologies, Warrenton, VA, USA). Multiple comparisons between means were performed using the Tukey test ($p \leq 0.05$). The influence of storage time on the physicochemical stability of emulsions by an unifactorial ANOVA test was also analyzed (95% level of confidence).

3. Results and discussion

3.1. Phospholipid composition

The phospholipid composition corresponding to the native and modified sunflower lecithins are shown in Table 1. As can be seen, the ethanol fractionation process was efficient, resulting in a phosphatidylcholine-enriched sunflower lecithin with twice higher PC percent and very lower PI content than the native. Besides, most of the triglycerides and free fatty acids from the native sunflower lecithin were removed by the deoiled process. The evidence of it was the marked increase in the total content of phospholipids in both deoiled and phosphatidylcholine enriched lecithins. These results are similar to those obtained by Cabezas et al. (2012) and Guiotto et al. (2013).

Table 1
Phospholipid composition of native and modified sunflower lecithins by ^{31}PNMR.

PL	NSL		DSL		PCL	
	g PL/ 100 g L	% of total PL	g PL/ 100 g L	% of total PL	g PL/ 100 g L	% of total PL
PC	14.25	36.66	20.83	35.82	49.98	78.33
1-LPC	nd	nd	0.10	0.17	0.22	0.34
2-LPC	0.51	1.31	0.97	1.67	2.03	3.18
PI	13.86	35.66	20.26	34.84	1.02	1.60
LPI	nd	nd	nd	nd	nd	nd
PS-Na	0.33	0.85	0.31	0.53	nd	nd
PE	4.15	10.68	6.90	11.87	4.75	7.44
LPE	0.16	0.41	0.30	0.52	0.29	0.45
APE	0.85	2.19	1.32	2.27	1.98	3.10
PG	0.34	0.87	0.56	0.96	0.67	1.05
DPG	0.68	1.75	0.99	1.70	0.06	0.09
PA	2.18	5.61	3.22	5.54	0.68	1.07
LPA	0.08	0.21	0.14	0.24	0.03	0.05
Other PL	1.48	3.81	2.25	3.87	2.10	3.29
Total	38.87	100	58.15	100	63.81	100

Average values (n = 2). Coefficient of variation < 4%.
nd = no signal assignment.
PL: Phospholipid NSL: Native sunflower lecithin; DSL: deoiled sunflower lecithin; PCL: Phosphatidylcholine-enriched sunflower lecithin PC: phosphatidylcholine; 1-LPC: 1-lysophosphatidylcholine; 2-LPC: 2-lysophosphatidylcholine; PI: phosphatidylinositol; LPI: lysophosphatidylinositol; PS-Na: phosphatidylserine; PE: phosphatidylethanolamine; LPE: lysophosphatidylethanolamine; APE: *N*-acyl-hosphatidylethanolamine; PG: phosphatidylglycerol; DPG: diphosphatidylglycerol; PA: phosphatidic acid; LPA: lysophosphatidic acid.

3.2. Characterization of O/W emulsions

3.2.1. Particle size

Fig. 2 shows the particle size distribution (PSD) of primary and secondary chia emulsions with differently modified lecithins in presence or absence of maltodextrin. At the initial time, the PSD curves corresponding to systems with deoiled lecithin had a monomodal shape for primary emulsions (L and L + M) (Fig. 2a). However, the peak corresponding to droplets with sizes between 0.1 and 1 μm decreased from a maximum of 11% (monolayer emulsions) to ~6–7% with the chitosan addition in bilayer systems. At the same time, a population of larger droplets appeared in PDS curves of these systems (Fig. 2b). Emulsions prepared only with phosphatidylcholine-enriched lecithin recorded a particle size distribution with a little shoulder of larger droplet sizes which was more noticeable in PC + M systems (Fig. 2c). The addition of chitosan in these systems led to trimodal curves with the appearance of a droplet population larger than 10 μm (Fig. 2d).

Regarding particle size, no significant ($p > 0.05$) differences were found in the droplet mean diameter $D_{3,2}$ using either deoiled or phosphatidylcholine-enriched sunflower lecithin (Table 2, Fig. 3a and c). These results are in agreement with Komaiko, Sastrosubroto, and McClements (2016) who reported no significant differences in the droplet diameter of fish O/W emulsions obtained by microfluidization with sunflower lecithins containing different amounts of phosphatidylcholine. However, other studies reported that lecithin enriched in phosphatidylcholine had better emulsifying properties and was more efficient for stabilizing lipid emulsions than deoiled one (Cabezas et al., 2012; Guiotto, Capitani, Nolasco, & Tomás, 2016; Guiotto et al., 2013). Thus, it is probable that also the type of homogenization process applied in each case, the electrical characteristics of the phospholipid head groups, the rheology of the emulsions obtained, have been key

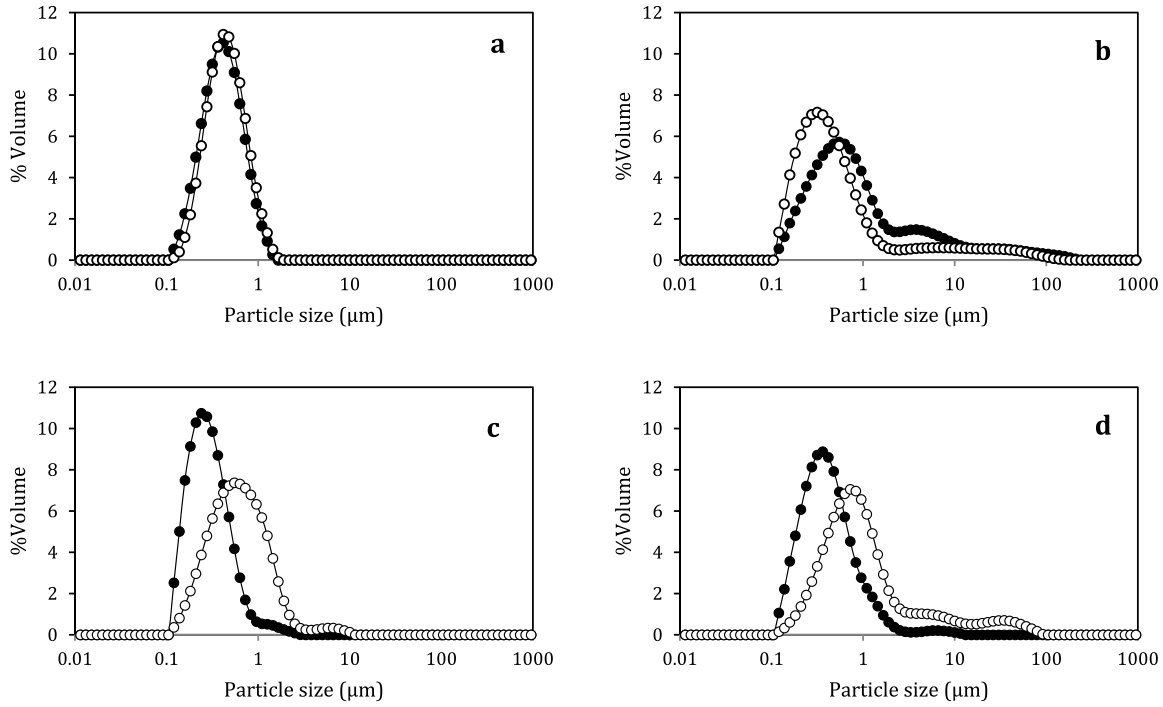

Fig. 2. Particle size distribution at initial time of deoiled (**a, b**) or phosphatidylcholine-enriched (**c, d**) sunflower lecithin stabilized by mono (**a, c**) and bilayer (**b, d**) chia O/W emulsions with (—○—) and without (—●—) maltodextrin. Average values are shown (n = 2).

factors in the performance of the different sunflower phospholipid emulsifiers.

With respect particle size displayed as the $D_{3,2}$ *surface*-weight diameter, it was significantly affected ($p \leq 0.05$) by the incorporation of chitosan and maltodextrin. Additionally, the interaction between modified lecithin type and maltodextrin addition was significant ($p \leq 0.05$) (Table 2). Both the addition of chitosan and maltodextrin led to larger particle sizes, which could be due to the formation of aggregates/flocs (Fig. 3a and b). Thus, a non-full chitosan coating on the lecithin-stabilized droplets may give rise to electrostatic attraction between the fully chitosan-lecithin coated droplets and those with "patches". Similar results were also found in β-carotene multilayer emulsions stabilized by soybean soluble polysaccharides and chitosan (Hou et al., 2010). Also, Fig. 3 shows the values of the mean diameter $D_{4,3}$ and Span for chia O/W emulsions stabilized with deoiled or phosphatidylcholine-enriched lecithin. In general, primary emulsions once formed presented values of $D_{4,3} \cong D_{3,2}$ while the secondary ones exhibited values of $D_{4,3} > D_{3,2}$. Besides, a higher polydispersity degree (Span) was observed for these last systems (Fig. 3a and b). These facts would suggest the presence of the aggregates/flocs.

No significant changes ($p > 0.05$) were recorded in the particle size during the storage time (data not shown).

3.2.2. ζ-potential

The charge on an emulsion droplet determines the nature of its interactions with other charged species including small ions, macromolecules or colloidal particles (McClements, 2007). The ζ-potential is the difference between the potential of the dispersion medium and the stationary fluid layer attached to the dispersed oil droplets. This parameter was measured to confirm the electrostatic deposition of chitosan on the interfacial film of deoiled or phosphatidylcholine-enriched lecithin that surrounds the droplets of chia oil and also to give information related to the emulsion stability. The results showed that the modified sunflower lecithin type, the chitosan addition and the

Table 2
Multifactorial analysis of variance (ANOVA) of the 3 × 2 fully factorial experimental design used to study the effect of sunflower modified lecithin type, chitosan and maltodextrin addition on the physicochemical properties of chia O/W emulsions.

Factor	Sum of square						
	Degrees of freedom	$D_{3,2}$	Span	ζ–potential	n	K *10^3	η_{100} * 10^3
Modified lecithin (A)	1	0.001	82.1833***	67.174***	0.003***	0.018	0.005
Chitosan addition (B)	1	0.066***	375.546***	30007.900***	0.013***	4.983***	1.387***
Maltodextrin addition (C)	1	0.037***	28.7135*	0.747	0.003***	0.997***	0.586***
AxB	1	0.0180	99.89***	50.057***	0.003***	0.019	0.005
AxC	1	0.057***	0.216225	2.320	0.000***	0.000	0.012*
BxC	1	0.000	20.8621*	0.239	0.003*	0.465***	0.192***
AxBxC	1	0.008*	0.036864	0.007	0.002***	0.000	0.007*
Pure error	8	0.009	24.0915	7.365	0.002	0.154	0.039
Total	15	1.179	631.539	30135.800	0.143	6.639	0.002

$D_{3,2}$ average oil droplet diameter (μm); Span; ζ–potential (mV); n flow behavior index; K consistency coefficient (Pa s^n); η_{100} apparent viscosity at 100 s^{-1}; PV peroxide value (meq hydroperoxide/kg oil).
*$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$.

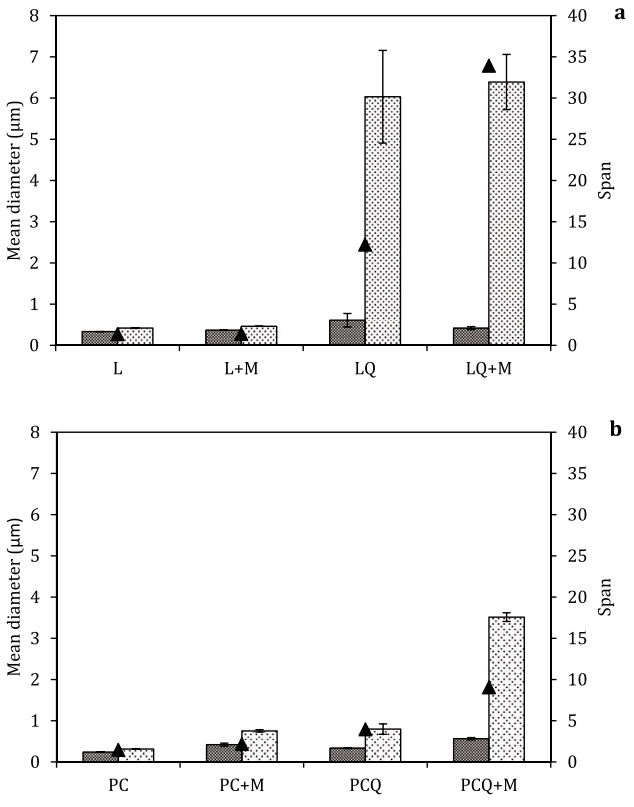

Fig. 3. Surface ($D_{3,2}$) (▨) and Volume ($D_{4,3}$) (⊡) mean diameters and polydispersity index (Span) (▲) of mono and bilayer chia O/W emulsions with deoiled (**a**) and phosphatidylcholine-enriched (**b**) sunflower lecithin. Average values are shown (n = 2).

interaction between both factors had a significant impact on the ζ-Potential (see Table 2). In systems without chitosan, the net charge of chia oil droplets was ~- 35–36 mV, because the modified sunflower lecithins used to stabilize these primary systems had a negative charge at pH 3, probably due to phosphate groups (PO^{3-}; $pK_a < 3.0$) (Table 3). This negative charge has been converted to high positive values ~ +47 (LQ, LQ + M) and ~ + 55 mV (PCQ, PCQ + M) by incorporating chitosan in the secondary emulsion systems, which would be indicative of the predominance of this cationic biopolymer on the surface of chia oil droplets, especially in emulsions with PC (Table 3). Thus, this charge inversion could be suggesting the deposition of cationic chitosan molecules with amine groups ($-NH^{3+}$; $pK_a < 6.3$) onto the anionic surface of the modified sunflower lecithin-coated oil droplets.

In general, systems with high ζ-potential absolute values (> +30 mV or < −30 mV) are very stable due to the strong repulsive electrostatic forces between the droplets (Wang, Li, Wang, & Adhikari, 2011). In this sense, it would be expected a high stability against aggregation by electrostatic repulsion arising from their electrical charge, mainly for bilayer emulsions.

3.2.3. Rheological properties

The rheological experimental results of fresh emulsions exhibited a high degree of fit to the Power Law model ($R^2 > 0.98$). Table 3 shows the apparent viscosity values at 100 s^{-1} (η_{100}), characteristics of processes such as agitation or chewing (McClements, 2005), and other rheological parameters (n: flow behavior index, K: consistency coefficient). Statistical analysis revealed that both chitosan and maltodextrin addition had a significant effect ($p \leq 0.05$) on all rheological parameters while the type of modified lecithin only affected the n factor. In turn, second and third level interactions were significant, being the "chitosan addition x maltodextrin addition" the most important (see Table 2). Bilayer systems, especially those with maltodextrin (LQ + M, PCQ + M), recorded higher levels of η_{100} ($p \leq 0.05$) than the monolayer ones (Table 3 and Fig. 4). Chitosan is known as a good viscosity-enhancing agent in acidic environments, due to its high molecular weight and linear unbranched structure (Xu, Aihemaiti, Cao, Teng, & Li, 2016). Thus, the presence of a large number of high molecular weight molecules into these systems tends to increase the resistance to flow which causes an increase in their apparent viscosity. In a similar trend, the maltodextrin addition induced a significant increase of the η_{100}, especially in secondary emulsions, which could be due to an increase of solid content in the continuous phase (Julio et al., 2016) (Table 3 and Fig. 4).

Moreover, according to the Power Law parameters, bilayer emulsions (LQ, LQ + M, PCQ, PCQ + M) recorded high consistency coefficients (K) and values of n < 1, which denotes a shear-thinning behavior probably associated with the presence of chitosan in the aqueous phase of these systems. For these emulsions, the increase in shear rate decreased the apparent viscosity which could be related to a continuous breakdown of the emulsion structure resulting in less resistance to flow. On the other hand, monolayer systems (L, L + M, PC, PC + M) exhibited low consistency coefficients (K) and values of n ~ 1, behaving as Newtonian fluids (Table 3 and Fig. 4).

3.3. Physical stability

The stability and shelf-life of emulsions depend on several factors, such as their viscosity, particle size, temperature, pH, and ionic forces (McClements, 2007). The backscattering (% BS) profiles constitute the macroscopic fingerprint of the emulsion at a given time (Mengual, Meunier, Cayré, Puech, & Snabre, 1999). Fig. 5 shows the BS profiles of the emulsions obtained as a function of the refrigerated storage for 50 days. Thus, BS profile evolution of the monolayer systems exhibited a destabilization process by creaming evidenced by the variation of the

Table 3
ζ-Potential, apparent viscosity at 100s^{-1} (η_{100}), and Power Law parameters (n, flow behavior index; K, consistency coefficient) for mono and bilayer chia O/W emulsions in presence or absence of maltodextrin at 25 ± 0.3 °C.

Systems	ζ-Potential (mV)	η_{100} (Pa.s^n).10^{-3}	Power Law parameters	
			n	K (Pa.s^n).10^{-3}
L	- 36 ± 0.2^a	1.54 ± 0.18^a	0.999 ± 0.001^d	1.54 ± 0.01^a
L + M	- 36 ± 0.9^a	4.94 ± 0.14^b	1.000 ± 0.001^d	4.95 ± 0.13^a
LQ	+47 ± 0.3^b	9.97 ± 0.81^c	0.837 ± 0.009^a	20.2 ± 1.29^b
LQ + M	+47 ± 0.8^b	21.24 + 0.39^d	0.866 ± 0.005^b	39.0 ± 1.63^c
PC	- 36 ± 0.2^a	1.26 + 0.10^a	0.999 ± 0.001^d	1.51 ± 0.06^a
PC + M	- 35 ± 0.1^a	5.15 + 0.66^b	0.998 ± 0.001^d	5.10 ± 0.57^a
PCQ	+54 ± 0.1^c	9.36 ± 0.23^c	0.866 ± 0.022^b	17.6 ± 0.59^b
PCQ + M	+55 ± 1.0^c	25.02 ± 2.50^e	0.923 ± 0.003^c	35.5 ± 3.78^c

*Different letters indicate significant differences ($p \leq 0.05$) in the same column.
Average values (n = 3) ± standard deviation.

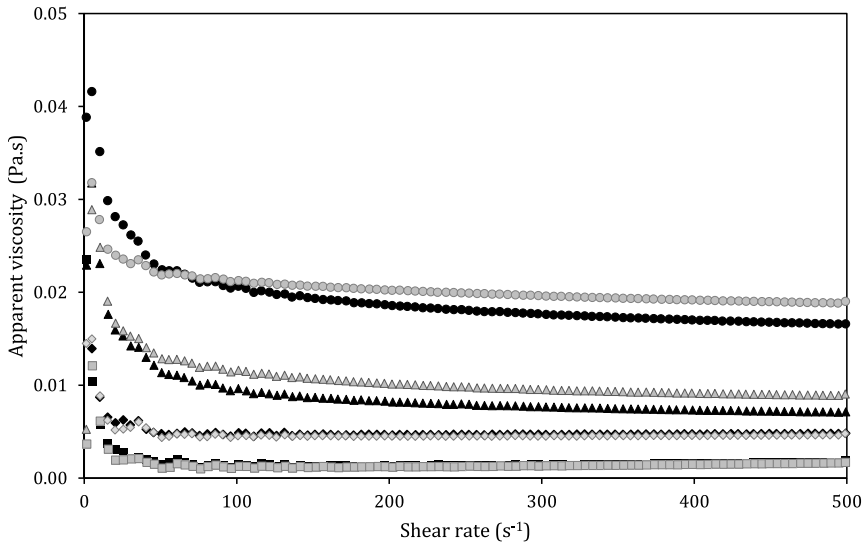

Fig. 4. Influence of chitosan deposition and maltodextrin addition on the apparent viscosity (η) of modified sunflower lecithin-stabilized emulsions (■) L (□), PC (◆), L + M (◇), PC + M (▲), LQ (△), PCQ (●), LQ + M (○), PCQ + M. Average values are shown (n = 3).

concentration of the droplets between the upper and the lower zones of the sample tube (Fig. 5a, b, e, f). In this sense, these systems recorded changes on their BS profiles in Zones I (~0–2.3 cm) and II (~5.2–6 cm) of the tube length after 5 d of storage. In Zone I, creaming destabilization is visualized by a gradual decrease of the initial %BS at the base of the tube as a function of storage time produced by the oil droplets upward movement due to their lower density in comparison to the surrounding liquid. Simultaneously, it appeared a peak in Zone II with % BS higher than the initial as result of a cream phase development with high concentration of oil droplets in the upper portion of the sample tube (Fig. 5a, b, e, f).

On the other hand, bilayer systems showed BS profiles without significant alterations which were indicative of a uniform oil droplet distribution along the sample tube during the storage period studied (Fig. 5c, d, g, h). This fact could be related to the high viscosity level of these emulsions as consequence of chitosan addition, which reduced the upward mobility of chia oil droplets according to Stokes' law. The chitosan deposition on modified sunflower lecithin-oil interfaces could stabilize these systems protecting them against creaming and coalescence as result of combined effects of the repulsion forces between the oil droplets and the viscosity enhancement described above. These results are in agreement with those reported by Xu et al. (2016) who found a significant improvement in the physical stability of lutein emulsions with the addition of 1 wt % of chitosan probably due to the formation of a gel network in these systems. Other researchers have described that the emulsion stabilization by using a combination of surfactant and polyelectrolytes or solid nanoparticles can synergistically enhance their stability (Binks, Rodrigues, & Frith, 2007; Stamkulov, Mussabekov, Aidarova, & Luckham, 2009).

In most of systems, no significant changes ($p > 0.05$) in the BS profiles with the addition of maltodextrin were found.

3.4. Oxidative stability

The lipid oxidation of the chia O/W emulsions was examined during their storage at 4 ± 1 °C without exposure to light for 50 days through their hydroperoxide and ω-3 fatty acid content. As can be observed in Fig. 6a, the initial low PV values obtained for all emulsions indicated that the emulsification process did not produce significant increases in primary oxidation products content. The hydroperoxide level of bilayer emulsions was low during the storage period (PV < 1 meq hydroperoxides/kg oil), recording final values lower ($p \leq 0.05$) than those corresponding to the monolayer systems (3–6 meq hydroperoxides/kg oil) and chia bulk oil (~2 meq hydroperoxides/kg oil). All emulsions presented lower levels of primary oxidation products than the upper limit (10 meq hydroperoxide/kg oil) established by the *Codex Alimentarius* (Codex Alimentarius Commission, 2012) for human consumption of oils not covered by individual standards (Fig. 6a). Additionally, ^{1}H NMR spectroscopy was used to monitor the ω-3 fatty acid content of these systems showing a good agreement with PV values. This technique has been reported as a good alternative to study the oxidative stability of emulsions, in terms of simplicity, specificity, and speed (Tyl, Brecker, & Wagner, 2008). The ω-3 fatty acid content of chia oil into emulsions was carried from the ^{1}H NMR spectra which provided qualitative and quantitative information about functional groups in the fatty acids. Some of these functional groups are points of attack in oxidation reactions, which are responsible for oxidative damage of the oils. Thus, from the spectra analysis and their changes during storage, the ω-3 fatty acid contents of different systems studied were analyzed (Fig. 6b). As can be seen, the monolayer emulsions recorded a higher loss of ω-3 fatty acid content in comparison with the bilayer emulsions. Therefore, the improvement in oxidative stability of two-layer systems could suggest that positively charged droplets (+47–55 mV) make it possible to decrease the interactions between the chia oil droplets with iron (Fe^{2+}, Fe^{3+}) -since they constitute pro-oxidant agents of O/W emulsions-by the existence of repulsive forces (Klinkesorn et al., 2005a). The higher stability exhibited by these systems vs. the primary ones could also be related to the greater thickness of the interfacial film of the same, which contributes to the reduction of the interactions previously discussed.

Regarding the secondary products of oxidation examined by TBARS test, low levels (< 1 mmol malonaldehyde equivalent/kg oil) were recorded for all systems studied, with no significant changes ($p > 0.05$) during the storage period (data not shown).

4. Conclusions

The present work provides information about two-layer chia O/W emulsions development by electrostatic deposition LBL technology, using natural emulsifiers (modified sunflower lecithins) and biopolymers (chitosan).

The electrostatic deposition of chitosan on the interfacial membranes, constituted by deoiled lecithin (DSL) or phosphatidylcholine-enriched (PCL) sunflower lecithin, could be evidenced by the charge inversion of the chia oil droplets. This fact had a strong influence on the emulsion characteristics leading to changes in the particle size, the

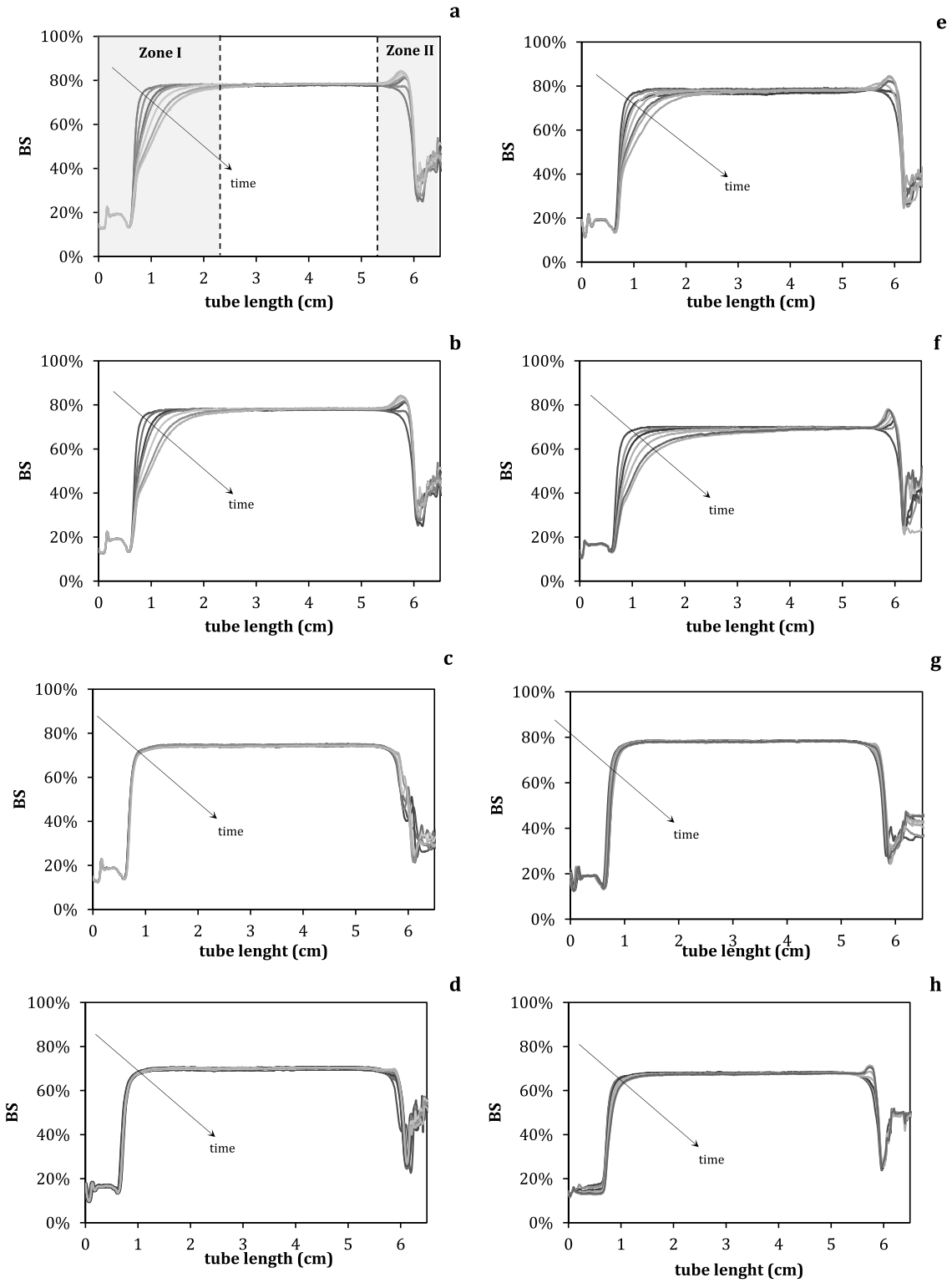

Fig. 5. Back Scattering (BS) profiles of the mono (**a, b, e, f**) and bilayer (**c, d, g, h**) chia O/W emulsions using deoiled (**a-c**) or phosphatidylcholine-enriched lecithin (**e-h**) with (**b, d, f, h**) and without (**a, c, e, g**) maltodextrin over 50 days of refrigerated storage. Each curve corresponds to a single time measurement (——): 0d (——), 5d (——), 10d (——), 16d (——), 25d (——), 35d (——), 45d and (——)50d.

rheological properties, and their storage stability. As for the overall stability, bilayer O/W emulsions recorded BS profiles with no significant alterations during the storage period, exhibiting a better physical stability than the monolayer ones. This behavior would be related to the higher viscosity level produced by the addition of chitosan in bilayer systems as well as the highly-charged interfaces of droplets. Regarding the oxidative stability, bilayer O/W emulsions were the most stable systems. The interfacial double-layer offered protection against lipid oxidation acting as a barrier against the penetration and the diffusion of prooxidant agents (transition metals) probably related to their greater thickness and the cationic nature of the oil droplets generating repulsion forces.

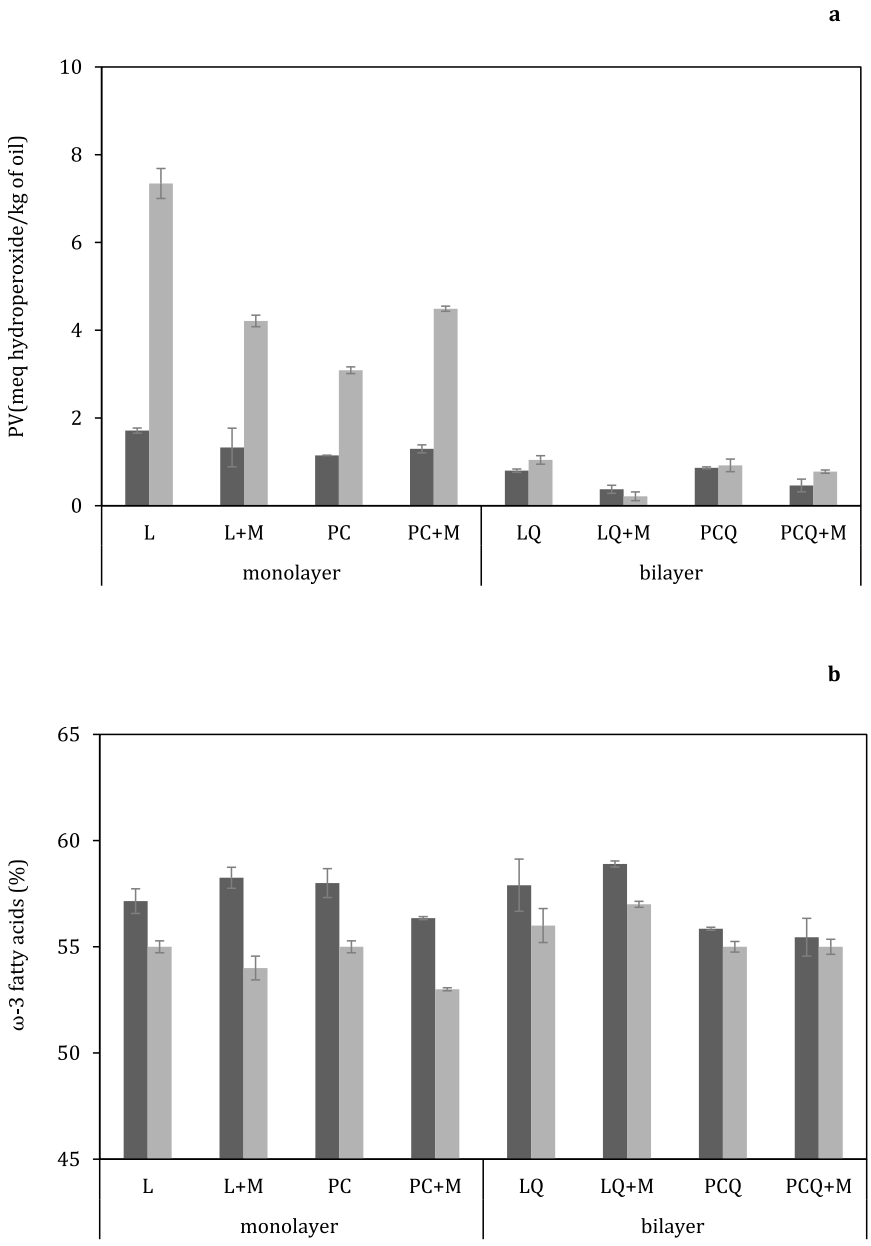

Fig. 6. Monitoring of lipid oxidation in mono and bilayer chia O/W emulsions by (**a**) hydroperoxides and (**b**) ω-3 fatty acid content at initial time (■)and after 50 days (■)of storage. Average values are shown (n = 2).

The maltodextrin addition affected mainly the droplet size and the rheological characteristics of emulsions. The systems formulated with this polysaccharide could be used to produce a powder product (microcapsules) from bilayer O/W emulsions drying.

According to these results, bilayer emulsions stabilized by modified sunflower lecithins-chitosan proved to be effective in protecting chia oil against lipid oxidation, keeping it stable for 50 days under refrigerated storage. Thus, these emulsions could be suitable delivery systems of omega-3 PUFAs from chia seed oil with potential application in the food industry.

Declaration of interest

The authors declare no conflict of interest.

Acknowledgments

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