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ORIGINAL PAPER

Stability of Oil-in-Water Emulsions with Sunflower (*Helianthus annuus* L.) and Chia (*Salvia hispanica* L.) By-Products

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Abstract Emulsifiers and stabilizers play an important role in emulsion stability. Optical characterization and droplet size distribution of oil-in-water emulsions formulated with different types and concentrations of modified sunflower lecithin [phosphatidylcholine (PC) enriched lecithin and deoiled sunflower lecithin], with or without chia mucilage (0.75 % wt/wt), have been evaluated as a function of storage time at 4 ± 1 °C. Emulsions with PC-enriched lecithin (without chia mucilage) exhibited the highest stability at the different concentrations because of the high PC/phosphatidylethanolamine ratio in comparison to Control lecithin. The addition of 0.75 % wt/wt mucilage contributed to obtain stable emulsions for all type and concentrations of emulsifiers studied, mainly with PC-enriched lecithin due to the reduction of the mobility of oil particles by the formation of a tridimensional network.

Keywords Modified sunflower lecithin · Chia mucilage · Oil-in-water emulsions · Storage conditions

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Introduction

Emulsions are heterogeneous systems composed of two-immiscible liquid phases (oil and water), with one of them dispersed as small droplets in the other. According to which phase makes up the droplets, there are two kinds of simple emulsions, oil-in-water (O/W) and water-in-oil (W/O). These are unstable systems from a thermodynamic point of view, but they may become kinetically stable by adding "emulsifiers" and/or "stabilizers" [1].

Vegetable oil blends are being developed to improve their nutritional profile. PUFA rich oils (mainly ω-3), such as chia seed oils can be blended with sunflower oil to obtain a balanced ω-6/ω-3 ratio according to FAO/WHO recommendation, presenting a high oxidative stability at low storage temperatures [2].

Emulsifiers, including lecithins, have hydrophilic (water soluble) and lipophilic (oil soluble) portions in their molecular structure, concentrate at the interface between oil and water and subsequently reduce the interfacial tension. Thereby, they facilitate the formation of an emulsion during the homogenization process [3, 4].

Sunflower lecithin is a by-product from crude oil refining obtained during the step called water-degumming. The distribution of the main phospholipid components of sunflower lecithin (16 % phosphatidylcholine (PC), 14 % phosphatidylinositol (PI), 8 % phosphatidylethanolamine (PE) appears to be rather similar to that of soybean lecithin (15 % PC, 10 % PI, 11 % PE) [5]. These amphipathic natural compounds are widely used in food products as emulsifiers, stabilizers, controlled-crystallization agents, viscosity modifiers, antioxidants, and reducers or replacers of fat [4].

Phospholipids, present a differential solubility in ethanol or acetone. This behavior has been used to obtain modified lecithins with different physicochemical and functional

properties which are desirable for different industrial purposes [6]. Deoiling consists of the separation of the insoluble polar lipids (glycolipids and phospholipids) and the soluble neutral lipids (triglycerides) with acetone [7]. On the other hand, PC is relatively more soluble in ethanol than PI; thus, an ethanol or ethanol-aqueous mixtures extraction gives rise to a PC enriched lecithin [6, 8]. Also, the PC/PE ratio can be used as a parameter related to potential properties as an O/W emulsifying agent, due to the different chemical structure, water interaction and geometric molecular behavior of the phospholipids at the interface. PC-enriched lecithin, due to its high PC/PE ratio and the lamellar phase structure of the PC at the interface between oil in water, is recognized as a good O/W emulsifier [7].

Stabilizers (mainly polysaccharides) are usually added to O/W food emulsions with low oil content, to obtain a stable system with good shelf life and to modify their textural properties. They provide long-term emulsion stability by immobilizing the droplets of the dispersed phase, increasing the viscosity [3]. In general, hydrocolloids are widely used in different applications at the food industry due to their ability to retain water. They are also notable for their thickening and gelling properties, syneresis control, emulsion stabilization, etc. [9].

Chia (*Salvia hispanica* L.) is a plant of the Lamiaceae family. In seeds of Lamiaceae, the mucilage consists of epidermal cells that swell or disintegrate into thin spiral fibrillae when they come into contact with water. After 5 min of soaking, it was observed that the cuticle had been broken and the exocarp cell content gradually surrounded the rest of the chia seeds. Chia mucilage is composed of -D-xylopyranosyl, -glucopyranosyl, and 4-*O*-methyl--D-glucopyranosyluronic acid unit in the ratio 2:1:1 [10]. The intake of chia mucilage—a type of soluble dietary fiber—has a positive effect on the metabolism of lipids. Mucilage forms gels of high viscosity producing gastric distension, feeling of satiety and slow stomach emptying, becoming a functional food [11]. Previous studies suggest that mucilage could be used as thickener in foods [12, 13], but there is no much information about its application in emulsions.

The aim of our research work was to study the effect of the addition of sunflower and chia by-products (modified lecithins, mucilage and sunflower-chia oil blends) on the physical stability of functional O/W emulsions as a function of storage time at 4 ± 1 °C.

Experimental Procedures

Materials

The native sunflower lecithin used as starting material was provided by a local oil producer (Vicentin SAIC-Rosario, Argentina).

The commercial chia seeds used in this study were obtained from Salta, Argentina (25ºS 65.5ºW). The seeds were manually cleaned and the foreign matter, such as granules, dirt and broken seeds, were removed. Afterwards, they were packaged in hermetic plastic containers and stored at 5 ± 1 °C until further use.

Chia and sunflower seed oils were provided by Nutracéutica Sturla SRL and Molinos Río de la Plata SA, Argentina. Chia oil obtained by cold-pressing of the seeds (without any further treatment) and refined sunflower oil were used.

Blending of Vegetable Oils

The oil blend was formulated by blending sunflower with chia seed oil in the proportion 80:20 wt/wt. The oils were thoroughly mixed for 5 min to obtain uniform blends, at room temperature (25 ± 1 °C).

Sunflower Lecithin Fractionation and Deoiling Process

The fractionation process was performed on 30 g of native sunflower lecithin with the addition of absolute-ethanol:water mixture 96:4 v/v, at a solvent extract:lecithin ratio of 3:1 (v/wt). Afterwards, the sample was incubated in a water bath at 65 °C for 90 min with moderate agitation (60 rpm), and then centrifuged at 1880 *g* and 10 °C for 10 min. The corresponding ethanolic extract and residues were obtained and ethanol was eliminated by evaporation under vacuum.

Ethanol soluble phase and native sunflower lecithin were deoiled with acetone, according to the official method Ja 4-46, procedures 1–5, of the American Oil Chemists' Society [14], to obtain the phosphatidylcholine enriched lecithin (PC-enriched lecithin) and deoiled sunflower lecithin (Control lecithin), respectively. Afterwards, both modified lecithins were stored at 0 °C. The fractionation procedure was performed in duplicate.

Phospholipid Composition

The phospholipid composition of the PC-enriched lecithins obtained as described above was determined by ^{31}P-NMR analysis in a Bruker Avance 600 MHz automatic spectrometer, with triphenyl phosphate as an internal standard (Spectral Service GmbH, Köln, Germany) [15]. For this purpose, 100 mg of this fraction was diluted in 1 mL of deuterated chloroform plus 1 mL of methanol and 1 mL of 0.2 M Cs-EDTA (pH 8.0). The organic layer was separated after a 15-min shaking, and analyzed by the above-mentioned spectroscopic technique. The determinations were performed in duplicate. The phospholipid composition of a given fraction was expressed as the molar concentration of

each PL class as a percent of the total PL molar concentration (i.e., %PC, %PI, and %PE).

Chia Mucilage

Mucilage was obtained by the procedure proposed by Marin Flores *et al.* [13], on a laboratory scale, with a modified drying method for the mucilage solution. Whole chia seeds were soaked in water (1:20 wt/v) for 1 h at room temperature with manual stirring in order to induce the mucilage exudation. The extracted mucilage was separated from the seeds by vacuum filtration through a mesh (100 μm) at 220 mbar. Afterwards, the mucilage solution was concentrated on a rotavapor (Buchi R-215, Switzerland) at 55 °C under vacuum. It was frozen at 20 °C for 96 h followed by freeze-drying (45 °C, 0.060 mbar, 5 days) (LABCONCO freeze dryer, Freezone 18, USA). The dried mucilage was ground using a food processor (Moulinex, model 1736249, Spain) to obtain a fine powder. Mucilage was packaged in hermetically sealed plastic containers, and stored in a desiccator to preserve it from humidity.

Proximate Composition of Mucilage

AOCS [14] procedures were used to analyze moisture (Ba 2a-38 method), crude fiber (Ba 6-84 method) and ash (Ba 5a-49 method) contents. Oil and nitrogen content (*N*) were determined following the IUPAC Standard Method [16] and the AOAC Method [17], respectively. Protein content was calculated as nitrogen × 6.25. Carbohydrate content was estimated as nitrogen-free extract (NFE) by difference using Eq. 1:

$$\text{NFE} = 100 - (\text{oil} + \text{protein} + \text{crude fiber} + \text{ash}) \quad (1)$$

The percentages of the different components were expressed on a dry basis (db).

Preparation of Aqueous Dispersions

The dispersions were prepared by adding 1.5 g dried mucilage to 80 g deionized water while stirring at 60 °C for 30 min. The dispersions were then cooled to room temperature and left overnight at 4 ± 1 °C (to ensure complete hydration) prior to use in emulsion preparation.

O/W Emulsion Preparation

O/W emulsions (10:90 wt/wt) were prepared by the addition of the different modified sunflower lecithins (PC-enriched lecithin, Control lecithin) in a range of 0.5–2.0 % (wt/wt) without and with chia mucilage (0.75 % wt/wt) respect to the aqueous phase, 10.0 % wt/wt of sunflower-chia oil blends and 0.01 % wt/wt of sodium azide to prevent bacterial growth. On the other hand, emulsions formulated with chia mucilage alone also contained a final concentration of 0.75 % wt/wt in the continuous phase.

The different emulsions were obtained at room temperature (25 ± 1 °C) in an Ultra-Turrax T-25 homogenizer (Janke and Kunkel, IKA-Labortechnik, Germany) using an S 25 N-10 G dispersing tool (rotor diameter 7.5 mm) at 10,000 rpm, for 1 min. The resulting pre-emulsions were then treated for 3.5 min with an ultrasonic processor (Sonics and Materials, Inc., USA) at full power (750 W). Once prepared, emulsions without and with chia mucilage were packaged in amber glass bottles (30 mL each) and stored at 4 ± 1 °C for 30 and 120 days, respectively. The pH of the emulsions was monitored periodically during storage. The experiments were performed in duplicate.

Rheological Properties

The flow behavior was analyzed with a Haake RS600 controlled stress rheometer (Thermo Electron, Germany) using a PP35-S serrated parallel plate measuring geometry (35 mm diameter), and measurements were performed at room temperature 25 ± 1 °C in the 1–500 s^{-1} range. All measurements were performed at initial time ($t = 0$) in triplicate. The experimental data (shear stress-shear rate) were fitted using the Power Law model according to Eq. 2:

$$\tau = k\,\dot{\gamma}^{n} \quad (2)$$

where τ is the shear stress (Pa); $\dot{\gamma}$ is the shear rate (s^{-1}); k the consistency coefficient (Pa·s^n) is the shear stress at a shear rate of 1.0 s^{-1} and the exponent n the flow behavior index, is dimensionless that reflects the closeness to Newtonian flow.

O/W Emulsion Stability

Physical stability of O/W emulsions was followed by measuring the variation of the percentage of backscattering (% *b*) *versus* the height of the sample (65 mm) using a Vertical Scan Analyzer (QuickScan, Beckman Coulter, Fullerton, USA), as described by Pan *et al.* [18]. The destabilization kinetics of the different emulsions was studied by recording the differential scanning profiles corresponding to the bottom zone I (10–15 mm) and the upper zone II (50–55 mm) of the tube as a function of storage time at 4 ± 1 °C. Measurements were performed immediately after preparation of emulsions and each week during the storage period. The experiments were performed in duplicate.

Particle Size Distribution

Particle size distribution was determined with a Malvern Mastersizer 2000E analyzer (Malvern Instruments Ltd., UK)

with a Hydro 2000MU dispersion unit. Optical parameters were refractive indexes of sunflower oil and water 1.47 and 1.33, respectively. Samples of each emulsion were diluted in a water container, at a stirring rate of 2000 rpm, in which a laser beam passed through a transparent internal cell where the diluted emulsion was recirculated. The light scattered at various angles from droplets of different sizes passed through a complex optical system, obtaining an angular light scattering pattern. The software of the equipment then translated this pattern into the corresponding particle size distribution [1]. The De Brouker mean diameter D (4, 3) associated with volume particle distribution was determined during storage time. The experiments were performed in duplicate.

Microscopic Observation

After preparation, the samples of each emulsion were observed with a Leica DMLB optical microscope. Micrographs were taken with a Leica DC 100 camera (Leica Microscopy Systems Ltd., Heerbrugg, Switzerland) with a magnification of 40×.

Statistical Analysis

Statistical analysis was performed by ANOVA at a 5 % significance level ($p \leq 0.05$). Means were separated according to Tukey's multiple comparison tests ($p \leq 0.05$) in all cases. Data were processed using the Statgraphics Plus statistical package (Version 4.0 for Windows, Manugistics Inc., USA) [19].

Results and Discussion

Phospholipid Composition of Modified Sunflower Lecithins

The phospholipid composition of native sunflower lecithin (SL), deoiled sunflower lecithin (control lecithin) and phosphatidylcholine enriched lecithin (PC-enriched lecithin) is shown in Table 1, where a marked difference can be seen between PC-enriched lecithin and control lecithin. PC-enriched lecithin exhibited the highest concentration of phosphatidylcholine (77.2 %) as well as lowest values of PI in comparison with SL and control lecithin, evidencing the efficiency of the fractionation process. These results are in agreement with those obtained by fractionation processes with absolute ethanol or ethanol–water mixtures [4, 7]. The PC-enriched lecithin showed a PC/PE ratio of 9.3, which is good level to favor its use as emulsifier agent in O/W emulsions instead of control lecithin and SL (3.1 and 3.2), respectively. These results are in agreement with those obtained by Guiotto *et al*. [7].

Table 1 Phospholipid (PL) composition (mol PL/100 mol lecithin) of modified sunflower lecithins determined by ^{31}P NMR

PL	PC-enriched lecithin	Control lecithin	SL
PC	77.2^{b}	36.4^{a}	37.0^{a}
PI	3.2^{a}	35.8^{b}	35.8^{b}
PE	8.3^{a}	11.6^{b}	11.6^{b}
PA	1.0^{a}	4.5^{b}	4.4^{b}
Others	10.5^{a}	11.7^{a}	11.1^{a}
PC/PE	9.3^{b}	3.1^{a}	3.2^{a}

Mean values followed by different letters differ significantly ($p < 0.05$) among rows, according to Tukey's test

SL native sunflower lecithin, *PL* phospholipid, *PC* phosphatidylcholine, *PI* phosphatidylinositol, *PE* phosphatidylethanolamine, *PA* phosphatidic acid

Chia Mucilage Composition

The mucilage extraction exhibited a yield of 3.5 ± 0.1 % (db) according to Capitani *et al.* [12]. The chia mucilage obtained presented the following proximal composition (db): moisture 9.37 %, protein 7.29 %, crude fiber 11.42 %, oil 3.83 %, ash 10.27 % and nitrogen-free extract (NFE) 56.24 %.

Rheological Properties

O/W emulsions without mucilage chia exhibited typical Newtonian flow behavior ($n = 1$), characterized by a linear relationship between the applied shear stress and the shear rate (i.e., constant viscosity) over the entire range of the parameters studied. The average viscosity (η) was of 0.0014 ± 0.0001 Pa·s, with no significant differences ($p > 0.05$) for the different types and concentrations of modified sunflower lecithin (PC-enriched lecithin, control lecithin). The parameter k is sometimes referred to as consistency index. For the case of a Newtonian fluid, the consistency index k is identically equal to the viscosity of the fluid, η.

Table 2 shows the power law parameters, consistency index (k) and flow behavior (n) of the upward curve of the O/W emulsions with chia mucilage. The coefficients of determination (R^2) were ≥0.9875 for all samples indicating that the power law model is adequate to describe the flow properties of emulsions with modified sunflower lecithins and chia mucilage. The k values for this type of emulsions varied between 0.680–0.720 and 0.549–0.565 Pa·s^n for PC-enriched lecithin and control lecithin, respectively. A low value of this coefficient was observed for emulsion with chia mucilage alone (0.411 Pa·s^n). Emulsions with chia mucilage and PC-enriched lecithin presented a more consistent index respect to the other ones; this fact could be related to a major PC-chia mucilage interaction. The

Table 2 Power law parameters of O/W emulsion with modified sunflower lecithins and chia mucilage

	Emulsifier concentration (% wt/wt)	k (Pa·s^n)	n	R^2
CM		0.411 ± 0.019^a	0.497 ± 0.012^b	0.9904
PC-enriched lecithin + CM	0.5	0.683 ± 0.012^c	0.385 ± 0.008^a	0.9875
	1.0	0.720 ± 0.029^c	0.366 ± 0.010^a	0.9901
	2.0	0.680 ± 0.031^c	0.370 ± 0.005^a	0.9889
Control lecithin + CM	0.5	0.559 ± 0.034^b	0.362 ± 0.004^a	0.9891
	1.0	0.565 ± 0.025^b	0.377 ± 0.007^a	0.9921
	2.0	0.549 ± 0.009^b	0.383 ± 0.009^a	0.9884

Mean values followed by different letters differ significantly ($p < 0.05$) among columns, according to Tukey's test

Average values ± SD ($n = 3$), *CM* Chia mucilage (0.75 % wt/wt); k consistency index, n flow behavior index (dimensionless)

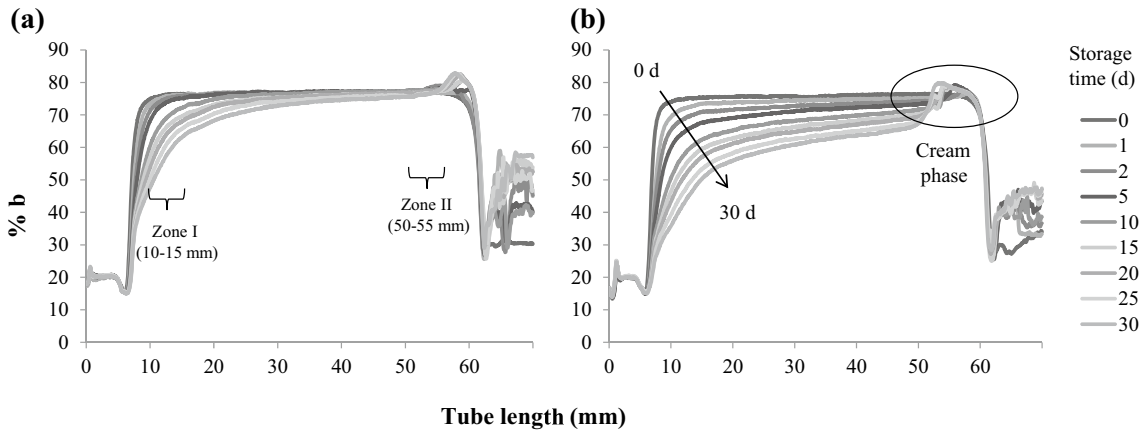

Fig. 1 Backscattering (% b) profiles of O/W emulsions with 1.0 % wt/wt of modified sunflower lecithins: **a** PC-enriched lecithin, **b** control lecithin as a function of storage time (d) at 4 ± 1 °C. Zone I (10–15 mm), zone II (50–55 mm). The length 0 mm was located at the bottom of the tube

flow behavior index (n) values were less than 1, indicating the pseudoplastic (shear thinning) nature of the emulsions. This parameter was lower for emulsions with PC-enriched lecithin or Control lecithin and chia mucilage *vs* systems constituted with chia mucilage alone. In addition, the n and k values did not exhibit significant differences ($p > 0.05$) as a function of emulsifier concentration. On the other hand, previous studies of emulsions with hydrocolloids suggest that the viscosity changed according to the predominant destabilization mechanism [20].

O/W Emulsion Optical Characterization

An average pH of 6.70 ± 0.17 was recorded for all emulsions during storage.

Figure 1 shows the % b profiles corresponding to emulsions containing 1.0 % of the modified sunflower lecithins (PC-enriched lecithin, control lecithin). The destabilization of the O/W emulsions was followed by monitoring the sequential profiles of the bottom of the tube, i.e., zone I (10–15 mm)—the area where a clarification of the emulsion is produced by the migration of the emulsified droplets to the top of the tube (i.e., creaming)—and the zone II (50–55 mm)—the portion where the potential destabilization of the cream phase occurs through coalescence process, whereby two or more droplets merge to form a single large droplet- [4, 7].

The comparative evolution of % b values corresponding to O/W emulsions containing different concentrations of modified sunflower lecithins (PC-enriched lecithin, control lecithin) can be visualized in Table 3. At the initial stage of storage ($t = 0$), the QuickScan profiles corresponding to zone I did not show significant variations ($p > 0.05$) of % b values between the different emulsions analyzed. During storage at 4 ± 1 °C, O/W emulsions with 0.5 and 1.0 % control lecithin presented a faster destabilization than the other ones. On the other hand, emulsion with 2.0 % of PC-enriched lecithin was the most stable system. After 30 d, emulsions with 0.5 and 1.0 % control lecithin showed a sharp decrease in % b of 40 and 38 %, respectively, while for 2.0 % of this emulsifier the decrease represented about 21 %. Emulsions with PC-enriched lecithin evidenced a

Table 3 Backscattering (% *b*) values of O/W emulsions with modified sunflower lecithins in a range of concentrations 0.5–2.0 % wt/wt (zone I 10–15 mm)

Time (days)	PC-enriched lecithin (% wt/wt)			Control lecithin (% wt/wt)		
	0.5	1.0	2.0	0.5	1.0	2.0
0	76.0 ± 0.7^{a}	75.4 ± 1.0^{a}	77.6 ± 0.7^{a}	74.9 ± 1.2^{a}	75.5 ± 0.8^{a}	77.5 ± 0.9^{a}
5	73.2 ± 1.0^{b}	73.5 ± 0.7^{b}	76.3 ± 0.6^{c}	64.8 ± 0.9^{a}	66.0 ± 0.6^{a}	77.5 ± 0.7^{c}
10	68.1 ± 0.7^{b}	70.7 ± 0.8^{c}	74.7 ± 0.7^{d}	58.0 ± 1.0^{a}	57.8 ± 1.1^{a}	73.1 ± 0.9^{d}
15	65.2 ± 0.9^{b}	68.4 ± 0.9^{c}	73.9 ± 0.8^{e}	54.5 ± 0.8^{a}	54.3 ± 0.9^{a}	70.6 ± 0.8^{d}
20	62.9 ± 0.6^{b}	66.9 ± 0.8^{c}	72.8 ± 0.9^{d}	51.5 ± 0.7^{a}	52.4 ± 0.9^{a}	68.0 ± 1.0^{c}
25	59.8 ± 0.8^{b}	64.0 ± 0.6^{c}	70.9 ± 1.0^{d}	47.3 ± 0.9^{a}	49.2 ± 0.8^{a}	62.4 ± 0.7^{c}
30	56.7 ± 0.8^{b}	61.6 ± 1.0^{c}	68.4 ± 0.7^{d}	44.8 ± 0.8^{a}	47.0 ± 0.9^{a}	61.6 ± 0.6^{c}

Mean values followed by different letters differ significantly ($p < 0.05$) among rows, according to Tukey's test

Average values ± SD ($n = 2$)

Table 4 Backscattering (% *b*) values of O/W emulsions with modified sunflower lecithins in a range of concentrations 0.5–2.0 % wt/wt and chia mucilage (0.75 % wt/wt) (zone I 10–15 mm)

Time (days)	PC-enriched lecithin + CM (% wt/wt)			Control lecithin + CM (% wt/wt)		
	0.5	1.0	2.0	0.5	1.0	2.0
0	70.8 ± 0.5^{a}	73.5 ± 0.4^{b}	76.1 ± 0.7^{c}	70.2 ± 0.2^{a}	72.3 ± 0.6^{b}	73.1 ± 0.5^{b}
21	70.4 ± 0.2^{a}	73.2 ± 0.5^{b}	75.5 ± 0.5^{c}	69.9 ± 0.5^{a}	72.2 ± 0.5^{b}	72.9 ± 0.6^{b}
42	70.4 ± 0.3^{a}	73.0 ± 0.5^{b}	75.2 ± 0.4^{c}	69.5 ± 0.3^{a}	72.1 ± 0.2^{b}	72.6 ± 0.5^{b}
63	70.0 ± 0.5^{a}	72.9 ± 0.3^{b}	75.4 ± 0.4^{c}	69.5 ± 0.4^{a}	72.0 ± 0.4^{b}	72.2 ± 0.5^{b}
84	69.6 ± 0.6^{a}	72.6 ± 0.4^{b}	75.1 ± 0.3^{c}	69.2 ± 0.4^{a}	71.6 ± 0.5^{b}	71.8 ± 0.3^{b}
105	69.0 ± 0.4^{a}	71.9 ± 0.6^{b}	74.9 ± 0.5^{c}	68.7 ± 0.6^{a}	71.4 ± 0.4^{b}	71.7 ± 0.4^{b}
120	69.1 ± 0.4^{a}	72.3 ± 0.5^{b}	74.4 ± 0.3^{c}	68.2 ± 0.5^{a}	71.3 ± 0.3^{b}	71.8 ± 0.5^{b}

Mean values followed by different letters differ significantly ($p < 0.05$) among rows, according to Tukey's test

Average values ± SD ($n = 2$), *CM* chia mucilage (0.75 % wt/wt)

significant increase ($p \leq 0.05$) of the emulsion stability as a function of increasing concentration. The influence of storage produced a decrease of the % *b* values (25, 18 and 12 %) for emulsions with 0.5, 1.0 and 2.0 %, respectively. It is interesting to note that the diminution of % *b* level at the bottom of the tube during storage can be related to the creaming process.

The zone II (50–55 mm) is characterized by the accumulation of oil droplets after the creaming process (cream phase). The difference of the density between the oil and water phases produces the migration of the oil droplets to the upper zone of the tube i.e., gravity separation. In this zone, it can generally be observed that the addition of both emulsifiers (PC-enriched lecithin, control lecithin) allowed the formation of a stable cream phase *versus* time during the studied storage period. Similar average % b for emulsions was recorded for the different emulsifiers (1.0, 2.0 %). However, emulsion with 0.5 % of control lecithin presented a significant decrease ($p \leq 0.05$) during the storage time (data not shown). This fact could be due that at this level of emulsifier the droplets were not protected by a sufficiently strong interfacial film. These results could be associated with the occurrence of the cream phase destabilization by coalescence.

The differences of the emulsifying properties of different modified sunflower lecithins may be related to the PC/PE ratio, since the PC-enriched lecithin is a better O/W emulsifier than Control lecithin [7]. On the other hand, in order to slow down the destabilization of emulsions, thickening agents such as polysaccharides and hydrocolloids are frequently used [20, 21].

In case of O/W emulsions with chia and sunflower by-products, the % *b* values corresponding to zone I (10–15 mm) showed an increase as a function of increasing concentration of modified lecithins, mainly with PC-enriched lecithin, while 1.0 and 2.0 % wt/wt control lecithin did not exhibit significant differences ($p \geq 0.05$) (Table 4). In particular, O/W emulsions with PC-enriched lecithin level of 2.0 % recorded higher % *b* values than those obtained with Control lecithin over the same concentration ($p \leq 0{,}05$), while for 0.5, 1.0 % wt/wt of these emulsifier, no significant differences ($p \geq 0.05$) were observed.

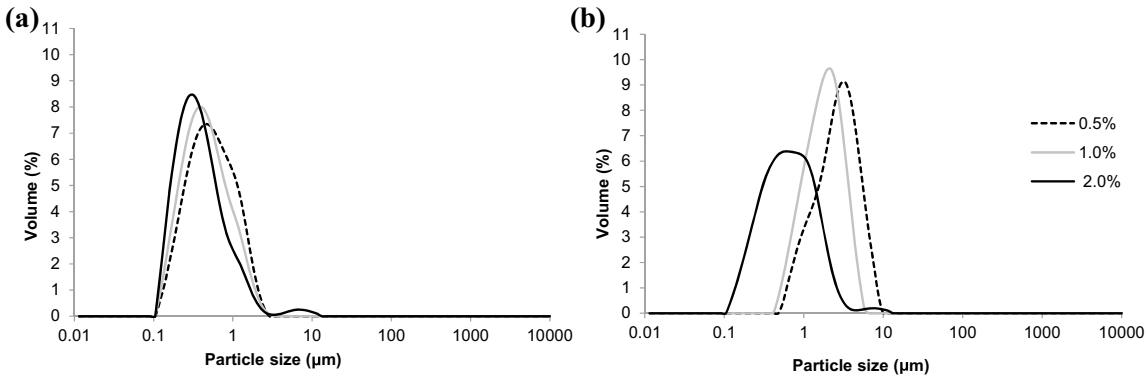

Fig. 2 Particle size distribution in volume of O/W emulsions with different concentrations (0.5–2.0 % wt/wt) of modified sunflower lecithins: **a** PC-enriched lecithin, **b** control lecithin at initial time ($t = 0$)

Table 5 De Brouker mean diameter D (4, 3) of O/W emulsions with modified sunflower lecithins during storage at 4 ± 1 °C

Time (days)		D (4,3) (μm)			
		0	7	15	30
PC-enriched lecithin (% wt/wt)	0.5	0.61 ± 0.02^{ab}	0.62 ± 0.02^{a}	0.57 ± 0.01^{a}	0.61 ± 0.03^{a}
	1.0	0.53 ± 0.03^{a}	0.61 ± 0.03^{a}	0.51 ± 0.03^{a}	0.53 ± 0.04^{a}
	2.0	0.47 ± 0.02^{a}	0.55 ± 0.01^{a}	0.53 ± 0.02^{a}	0.54 ± 0.02^{a}
Control lecithin (% wt/wt)	0.5	1.72 ± 0.05^{d}	2.51 ± 0.04^{d}	2.63 ± 0.02^{d}	2.82 ± 0.05^{d}
	1.0	1.39 ± 0.03^{c}	1.92 ± 0.04^{c}	1.90 ± 0.03^{c}	1.87 ± 0.04^{c}
	2.0	0.69 ± 0.01^{b}	0.78 ± 0.02^{b}	0.81 ± 0.02^{b}	0.81 ± 0.01^{b}

Mean values followed by different letters differ significantly ($p < 0.05$) among columns, according to Tukey's test

Average values ± SD ($n = 2$)

The % b values of the cream phase (zone II) of these O/W emulsions showed a similar behavior to that observed in the zone I (data not shown). All emulsions remained stable during storage at 4 ± 1 °C, being the average % b with 0.5, 1.0 and 2.0 % of PC-enriched lecithin 69.9 ± 0.6, 72.7 ± 0.4 and 75.5 ± 0.3, respectively instead of 69.2 ± 0.5, 71.9 ± 0.4 and 72.2 ± 0.6, respectively in case of control lecithin.

It is worth mentioning that the O/W emulsions formulated with different concentrations of modified sunflower lecithin (PC-enriched lecithin, control lecithin) and chia mucilage (0.75 % wt/wt) exhibited similar profiles with higher stability against creaming throughout the period analyzed (120 days) (Table 4) than the emulsions previously described (30 days, see Table 3). This fact could be mainly attributed to the addition of chia mucilage which would prevent the individual droplets for moving, and therefore, would decrease the probability of formation of aggregates/fusion of droplets contributing to the emulsion stability. Similar results were reported for O/W emulsions formulated with different concentrations of other hydrocolloids [20, 21]. Emulsions with chia mucilage alone presented a good stability during the storage period with % b levels of 70 %. Avila-de la Rosa *et al*. [22] studied the viscoelastic response of chia seed mucilage in the vicinity of the oil–water interface observing that their emulsification properties arise from the formation of spatial structures surrounding the oily phase.

Particle Size Distribution

Figure 2 shows the comparative particle size distribution in volume for O/W emulsions obtained with different modified sunflower lecithins (PC-enriched lecithin, control lecithin) and concentrations (0.5–2.0 % wt/wt) at initial time ($t = 0$). These distributions presented a monomodal or bimodal character depending on the concentration of modified lecithin added. In this sense, 2.0 % modified lecithin (PC-enriched lecithin, control lecithin) showed a bimodal character, but these small populations of large particles did not affect the stability of emulsions. Regarding emulsions with Control lecithin, it was possible to observe a displacement to a smaller droplet size as a function of increasing concentrations.

O/W emulsions formulated with PC-enriched lecithin presented similar mean diameter D (4, 3) values and no

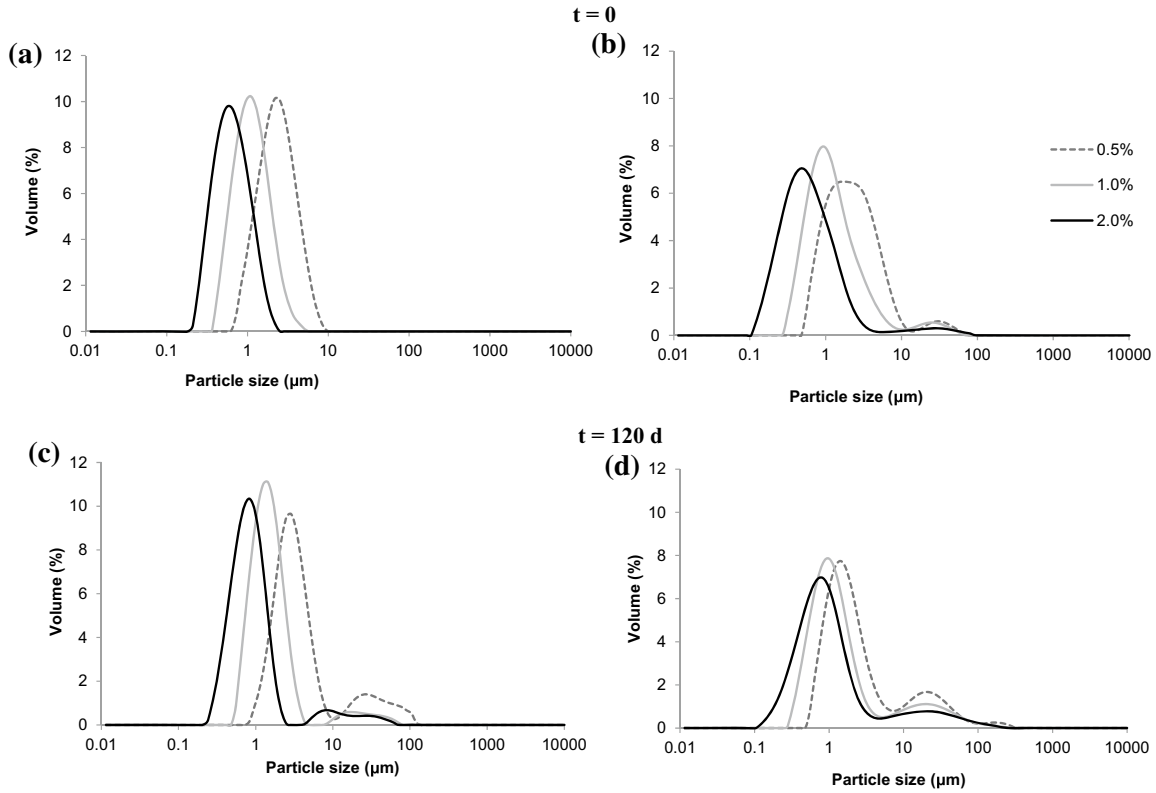

Fig. 3 Particle size distribution in volume of O/W emulsions with different concentrations (0.5–2.0 % wt/wt) of modified sunflower lecithins and chia mucilage (0.75 % wt/wt): **a**, **c** PC-enriched lecithin; **b**, **d** control lecithin at initial ($t = 0$) and final storage time ($t = 120$ days)

Table 6 De Brouker mean diameter *D* (4,3) of O/W emulsions with modified sunflower lecithins and chia mucilage during storage at 4 ± 1 °C

Time (days)		0	30	60	90	120
	CM	13.51 ± 0.40^{d}	13.88 ± 0.20^{d}	15.78 ± 0.02^{e}	16.28 ± 0.15^{e}	16.53 ± 0.35^{e}
PC-enriched lecithin (% wt/wt)	0.5 + CM	2.24 ± 0.29^{ab}	9.73 ± 0.33^{c}	9.75 ± 0.35^{d}	9.46 ± 0.56^{d}	9.17 ± 0.11^{d}
	1.0 + CM	1.90 ± 0.06^{a}	5.60 ± 0.28^{b}	5.77 ± 0.27^{bc}	5.36 ± 0.39^{c}	4.64 ± 0.19^{b}
	2.0 + CM	1.52 ± 0.16^{a}	3.76 ± 0.22^{a}	3.01 ± 0.40^{a}	3.02 ± 0.18^{a}	2.58 ± 0.10^{a}
Control lecithin (% wt/wt)	0.5 + CM	4.90 ± 0.30^{c}	9.15 ± 0.14^{c}	9.43 ± 0.29^{d}	8.49 ± 0.13^{d}	8.82 ± 0.11^{d}
	1.0 + CM	2.97 ± 0.31^{b}	5.61 ± 0.13^{b}	6.54 ± 0.18^{c}	6.05 ± 0.27^{c}	6.26 ± 0.02^{c}
	2.0 + CM	1.86 ± 0.19^{a}	4.35 ± 0.41^{a}	4.77 ± 0.13^{b}	4.30 ± 0.04^{b}	4.91 ± 0.32^{b}

Mean values followed by different letters differ significantly ($p < 0.05$) among columns, according to Tukey's test

Average values ± SD ($n = 2$); *CM* Chia mucilage (0.75 % wt/wt)

significant differences ($p > 0.05$) were recorded between the different concentrations (Table 5). O/W emulsions with control lecithin showed a significant decrease ($p \leq 0.05$) of mean diameter *D* (4, 3) values as a function of increasing concentration of this emulsifier agent. Emulsions with 2.0 % of control lecithin exhibited mean diameter *D* (4, 3) values between 0.69 and 0.81 μm, without significant differences ($p > 0.05$) during refrigerated storage time. Furthermore, emulsions with 0.5 and 1.0 % of control lecithin, increased significantly ($p \leq 0.05$) compared to the corresponding mean diameter *D* (4, 3) values at the initial time (statistical data not shown) (Table 5).

D (4, 3) values correlate with the destabilization shown in Table 3, in which the decrease of % *b* values was mainly

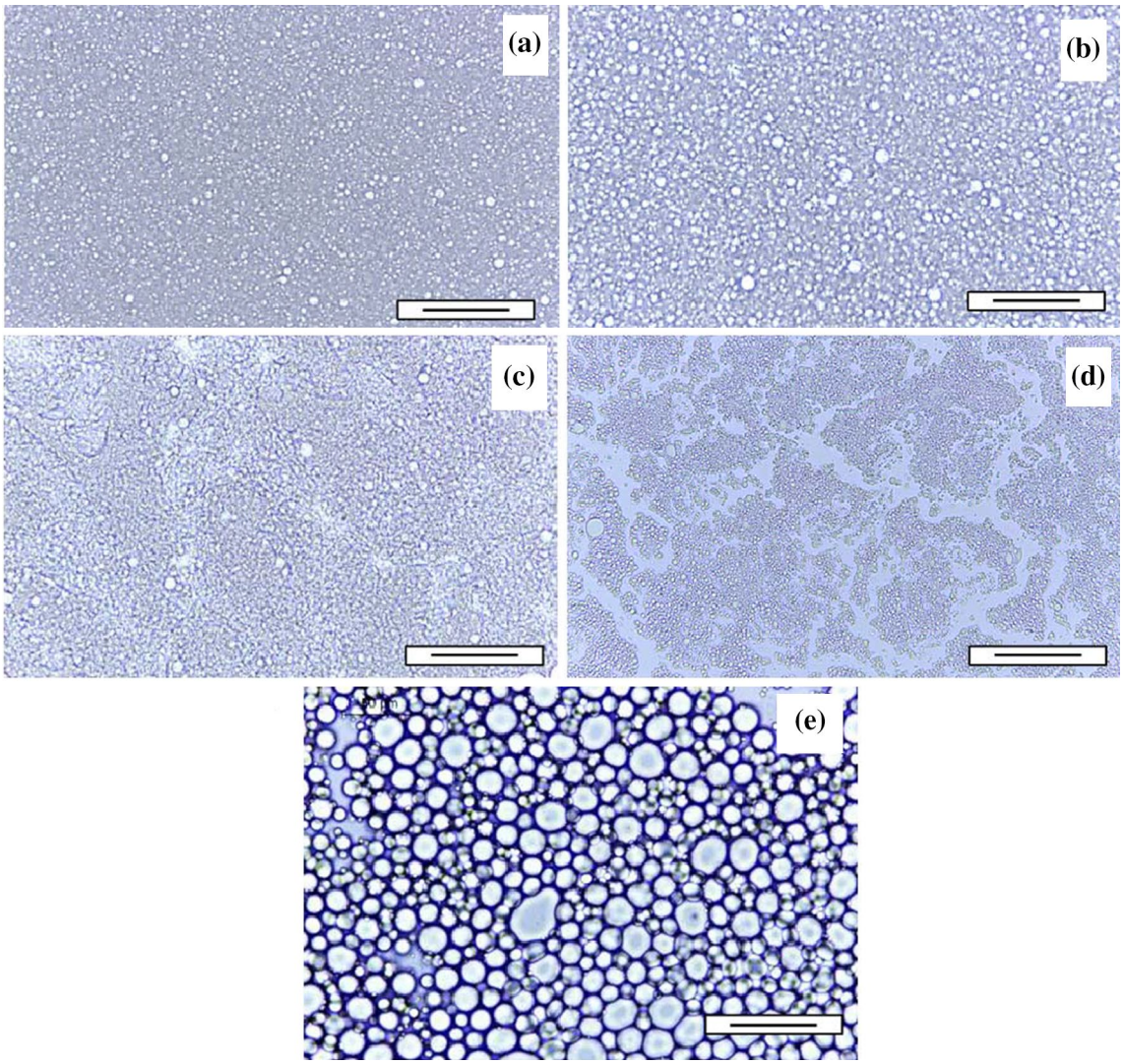

Fig. 4 Optical micrographs of O/W emulsions with 2.0 % wt/wt of modified sunflower lecithins: **a** PC-enriched lecithin; **b** control lecithin and modified sunflower lecithins–chia mucilage (0.75 % wt/wt): **c** PC-enriched lecithin; **d** control lecithin; **e** chia mucilage alone at initial time ($t = 0$). *Black bars* 50 μm

associated with a migration of particles (gravity separation) rather than a droplet size increase (coalescence). Furthermore, emulsions with PC-enriched lecithin were more stable due to the formation of droplets of smaller diameter with respect to emulsions with Control lecithin.

The particle size distributions of O/W emulsions with modified sunflower lecithins (PC-enriched lecithin, Control lecithin) and chia mucilage at initial and final time of storage are shown in Fig. 3. A displacement of the curves toward to the right (large droplets) can be seen as a function of decreased concentration of these emulsifier agents.

At the initial time, the particle size distribution of the O/W emulsions with different concentrations of PC-enriched lecithin and chia mucilage exhibited monomodal character. Moreover, the emulsions made with Control lecithin and chia mucilage, showed bimodal character for all concentrations.

During the storage time it was possible to observe the evolution of the particle size distribution from a monomodal to a bimodal profile for O/W emulsions with PC-enriched lecithin and chia mucilage, whereas for emulsions with Control lecithin and chia mucilage the larger droplet population was increased. These changes observed in particle size distribution did not significantly influence the physical stability of the O/W emulsions.

Table 6 shows that D (4, 3) values decreased significantly ($p \leq 0.05$) as a function of enhancing modified sunflower lecithin concentration with chia mucilage. The D (4, 3) values of emulsions formulated with chia mucilage alone were higher than other ones and exhibited an

increasing tendency as a function of storage time (statistical data not shown). Regarding refrigerated storage time, the *D* (4, 3) values of different O/W emulsions showed significant increases ($p \leq 0.05$) over the first 30 days and then remained stable (Table 6). This fact was evidenced in the particle size distributions through the formation and/or increase of a second population of large droplets (see Fig. 3).

On the other hand, the *D* (4, 3) values for O/W emulsions with 0.5 % of emulsifiers did not show significant differences ($p > 0.05$) between PC-enriched and control lecithin, both with CM from 30 days of storage. O/W emulsion with levels of 1.0 % PC-enriched lecithin exhibited *D* (4, 3) values significantly lower ($p \leq 0.05$) with respect to those with Control lecithin for 0 and 120 days. At levels of 2.0 %, from 60 days of storage, *D* (4, 3) values of the O/W emulsion with PC-enriched lecithin were significantly lower ($p \leq 0.05$) compared to the emulsions with Control lecithin (Table 6).

The comparative evaluation of O/W emulsions with chia mucilage alone *vs* those with modified sunflower lecithins (PC-enriched lecithin, control lecithin) and chia mucilage (0.75 % wt/wt) suggests a significant effect of the presence of PC-enriched or control lecithin on the particle size. The chia mucilage—modified lecithin interaction could favor the production of particles of smaller diameter, higher interfacial area than those emulsions without this chia by-product.

Microscopic Observation

Figure 4a, b shows the optical micrographs of O/W emulsions with 2.0 % wt/wt of modified sunflower lecithins (PC-enriched lecithin, Control lecithin), i.e., oil droplets dispersed in the aqueous phase. The provided information was in accordance with the results obtained by the particle size distribution and optical characterization. Similar images were observed at other concentrations (0.5 and 1.0 % wt/wt) for both emulsifiers (data not shown).

The simultaneous addition of chia mucilage and modified sunflower lecithin (2.0 % wt/wt) in emulsions evidenced their compact or structured appearance (Fig. 4c–d). This fact could be associated with the formation of a tridimensional network which is maintained within the oil droplets. It is interesting to observe the particular characteristics exhibited by O/W emulsions with chia mucilage alone (Fig. 4e) which are also in agreement with the experimental results previously discussed.

Conclusions

O/W emulsions with PC-enriched fractions exhibited higher stability in comparison with those obtained with control lecithin. This fact could be associated with the highest PC/PE ratio of the PC-enriched lecithin, which would favor their use as an emulsifier agent in this type of emulsion. Chia mucilage presented a good performance as a functional ingredient to be used as stabilizer or thickener agent in emulsions. The addition of 0.75 % wt/wt mucilage contributed to obtain emulsions stable against creaming during refrigerated storage due to the reduction of the mobility of oil particles by the formation of a tridimensional network. Modified sunflower lecithin and chia mucilage interaction would favor the production of stable emulsions, which present high % *b* values and low particle sizes as a function of increasing concentration of emulsifier, mainly with PC-enriched lecithin.

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