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# Emulsifying Properties of Different Modified Sunflower Lecithins

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**Abstract** Lecithins are a mixture of acetone-insoluble phospholipids and other minor substances (triglycerides, carbohydrates, etc.). The most commonly processes used for lecithin modification are: fractionation by deoiling to separate oil from phospholipids, fractionation with solvents to produce fractions enriched in specific phospholipids, and introduction of enzymatic and chemical changes in phospholipid molecules. The aim of this work was to evaluate the emulsifying properties of different modified sunflower lecithins in oil-in-water (O/W) emulsions. In this study, five modified sunflower lecithins were assessed, which were obtained by deoiling (deoiled lecithin), fractionation with absolute ethanol (PC and PI enriched fractions), and enzymatic hydrolysis with phospholipase $A_2$ from pancreatic porcine and microbial sources (hydrolyzed lecithins). Modified lecithins were applied as an emulsifying agent in O/W emulsions (30:70 wt/wt), ranging 0.1–2.0% (wt/wt). Stability of different emulsions was evaluated through the evolution of backscattering profiles (%BS), particle size distribution, and mean particle diameters (D [3, 4], D [3, 2]). PC enriched fraction and both hydrolyzed lecithins presented the best emulsifying properties against the main destabilization processes (creaming and coalescence) for the analyzed emulsions. These modified lecithins represent a good alternative for the production of new bioactive agents.

**Keywords** Sunflower lecithin · Fractionation process · Enzymatic hydrolysis · O/W emulsions · Phospholipids · $^{31}$P NMR

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## Introduction

Lecithins have been widely used in nutritional, pharmaceutical and cosmetic industries [1–3]. The food industry mainly includes natural or modified lecithins in many processes due to their versatile role as emulsifiers, viscosity regulators, anti-spattering and dispersing agents [4–6].

Emulsifiers, including lecithins with hydrophilic and lipophilic portions in their molecular structure, concentrate at the interface between oil and water and subsequently reduce the interfacial tension. Then, emulsifiers facilitate the formation of an emulsion during the homogenization process. Also, these compounds prevent destabilizing processes such as creaming, coalescence, sedimentation and separation, thus increase the shelf life of the product [7, 8]. In particular, surface activity and performance of commercial lecithin can be improved by modification processes such as fractionation with alcohols or enzymatic changes in the phospholipid structure, as other authors and patents have described [9–11].

The main components of native lecithins, i.e., phospholipids, present a differential solubility in ethanol or acetone. This behavior has been used to obtain fractions with different phospholipid composition compared to the

starting material [12–14]. Deoiling can be considered as the most important fractionation process for production of lecithin powder, based on the separation of the insoluble polar lipids (glycolipids and phospholipids) and the soluble neutral lipids (triglycerides) with acetone [15–17]. On the other hand, phosphatidylcholine (PC) dissolves better and faster than the other phospholipids in alcohols. Therefore, several alcohols in different concentrations can be used to obtain an ethanol soluble fraction containing a high PC/PE (phosphatidylethanolamine) ratio, but an insoluble fraction with low PC/PE ratio [13, 18, 19].

Enzymatic hydrolyzed lecithins may present technological and commercial advantages over native lecithins; consequently, the demand for lysolecithins has been increasing in recent years [20]. Phospholipase $A_2$ ($PLA_2$) is the most employed family of enzymes for the enzymatic modification of lecithin. Porcine pancreatic $PLA_2$ has been used on an industrial scale for decades, e.g., in the production of food products such as mayonnaise and degumming of edible oils. With the development of biotechnology, a number of phospholipases were expressed in hosts other than the native ones (fungi, microbes, plants). This fact has led to the production of new phospholipases and the development of novel industrial applications [11, 21].

In Argentina, the production of sunflower oil is of utmost importance from an economic point of view [22]. Sunflower lecithin, i.e., a byproduct of the degumming process of oils [17, 23], is an interesting alternative to soybean lecithin because it is considered as a non-GMO (non-genetically modified organisms) product, which is currently preferred by certain consumers.

The objective of this work was to evaluate the emulsifying properties of different modified sunflower lecithins applied in O/W emulsions. With this aim, several modification processes were applied to sunflower lecithin, i.e., deoiling, fractionation and enzymatic hydrolysis. In this sense, this study seeks to contribute to the food industry with useful information about developing tailor-made surface-active emulsifiers.

## Materials and Methods

### Materials

Native sunflower lecithin was used as the starting material, and was provided by a local oil industry (Vicentin S.A.I.C.). Enzymatic hydrolysis processes were carried out using a porcine pancreatic $PLA_2$ (Lecitase 10L, Novo Nordisk) and a microbial $PLA_2$ (*Streptomyces violaceoruber*, LysoMax $PLA_2$, Danisco). All solvents used were of analytical grade.

Native sunflower lecithin was deoiled with acetone, according to AOCS official method Ja 4-46, procedures 1–5 [14, 24], obtaining the deoiled sunflower lecithin (DSL). Then, DSL was stored at 0 °C. DSL, without modification by fractionation or hydrolysis, was used as sample control. The deoiling procedure was performed in duplicate.

### Lecithin Fractionation

Fractionation process was performed with 30 g of native sunflower lecithin with the addition of absolute ethanol (ethanol/lecithin ratio 3:1). This sample was incubated in a water bath at 65 °C during 90 min with moderate agitation (60 rpm), and then centrifuged at 1880 g and 10 °C for 10 min. Afterwards, the corresponding ethanolic extracts and residues were obtained and ethanol was eliminated by evaporation under vacuum.

Ethanol soluble and insoluble phases (residues) were further deoiled with acetone, obtaining the PC and PI (phosphatidylinositol) enriched fractions, respectively. Then, both fractions were stored at 0 °C. The fractionation procedure was performed in duplicate.

### Lecithin Hydrolysis

Enzymatic hydrolysis was carried out in a thermostated reactor on a laboratory scale, using 27 g of native sunflower lecithin and 18 ml of 0.4 M $CaCl_2$. The initial pH was adjusted to 7 by adding 4 N NaOH solution. Then, the resulting mixture was set to the optimal temperature of each phospholipase, i.e., 60 °C for porcine pancreatic $PLA_2$ and 50 °C for microbial $PLA_2$, which were incorporated in a concentration of 2.0% ml lipase per 100 g lecithin. Next, continuous agitation (50 rpm) was applied for 5 h. Evolution of hydrolysis process was followed by measuring pH, using a pH meter for solid samples (840049 Puncture Tip, Saen S.R.L.) [20, 25]. Hydrolysis products were also deoiled using acetone and then stored at 0 °C. Enzymatic hydrolysis was carried out in duplicate, for each case.

### Phospholipid Composition

Phospholipid composition of samples obtained after different modification processes was determined by $^{31}$P-NMR analysis in a Bruker Avance 600 MHz automatic spectrometer, using triphenyl phosphate as the internal standard (Spectral Service GmbH, Köln, Germany) [26]. For this purpose, 100 mg of each modified lecithin was diluted in 1 ml of deuterated chloroform, 1 ml of methanol and 1 ml of 0.2 M Cs-EDTA (pH 8.0). The organic layer was separated after 15 min of shaking, and analyzed by the described spectroscopic technique. Phospholipid composition was

**Table 1** Phospholipid (PL) composition of modified sunflower lecithins by $^{31}$P NMR[a]

| | Phospholipid weight division (%) | | | | | Phospholipid mol division (%) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | DSL | PCF | PIF | SHLP | SHLM | DSL | PCF | PIF | SHLP | SHLM |
| PC | 21.8 | 44.3 | 12.0 | <0.1 | 4.7 | 35.1 | 75.4 | 19.0 | <0.1 | 6.8 |
| 1-LPC | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 |
| 2-LPC | 0.8 | 1.7 | 1.1 | 23.4 | 16.8 | 2.0 | 4.3 | 2.6 | 46.1 | 36.4 |
| PI | 25.5 | 2.7 | 35.2 | <0.1 | 11.4 | 37.8 | 4.2 | 51.3 | <0.1 | 15.2 |
| LPI | <0.1 | <0.1 | <0.1 | 16.2 | 8.2 | <0.1 | <0.1 | <0.1 | 28.8 | 16.1 |
| PE | 8.3 | 5.6 | 9.4 | 0.5 | 4.3 | 14.1 | 10.1 | 15.7 | 0.6 | 6.6 |
| LPE | 0.3 | 0.2 | 0.4 | 5.9 | 3.6 | 0.7 | 0.6 | 1.0 | 12.8 | 8.5 |
| APE | 1.6 | 2.2 | 1.3 | <0.1 | <0.1 | 2.0 | 2.9 | 1.6 | <0.1 | <0.1 |
| PA | 2.3 | 0.4 | 3.0 | <0.1 | 2.1 | 4.1 | 0.8 | 5.4 | <0.1 | 3.4 |
| LPA | <0.1 | <0.1 | <0.1 | 1.8 | 0.3 | 0.1 | <0.1 | 0.2 | 4.2 | 0.7 |
| Other | 2.4 | 0.9 | 2.0 | 5.7 | 4.4 | 4.1 | 1.7 | 3.4 | 7.5 | 6.3 |
| Sum | 62.8 | 57.9 | 64.4 | 53.4 | 55.6 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |

[a] Average values are shown ($n = 2$). The coefficient of variation was lower than 5%

expressed in terms of phospholipid weight concentration (g PL/100 g lecithin) and molar concentration (mol PL/100 mol Total PL) (Table 1).

### Oil-in-Water (O/W) Emulsions Preparation

Commercial sunflower oil was used to prepare oil-in-water (O/W) emulsions with a formulation of 30:70 wt/wt. Emulsions were prepared at room temperature in an Ultra-Turrax T25 homogenizer using S 25 N–10 G dispersing tool (7.5 mm rotor diameter) at 10,000 rpm for 1 min, according to Pan et al. [27], with the addition of the different modified sunflower lecithins in a range of 0.1–2.0% (wt/wt).

### Optical Characterization of Emulsions

The backscattering of light was measured using a Quick Scan Vertical Scan Analyzer (Coulter Corp., Miami, FL). The backscattering of monochromatic light ($\lambda = 850$ nm) from the emulsions was determined as a function of the height of the sample tube (ca. 65 mm) in order to quantify the rate of different destabilization processes during the first 90 min. This methodology allowed discrimination between particle migration (sedimentation, creaming) and particle size variation (flocculation, coalescence) processes [23]. The basis of the multiple light scattering theory has been exhaustively studied by Mengual et al. [28].

### Particle Size Measurements

Particle size distribution, and De Brouckere (D [4, 3]) and Sauter (D [3, 2]) mean diameters of particles of the emulsions were determined with a particle size analyzer (Malvern Mastersizer 2000E, Malvern Instruments Ltd., Worcestershire, UK). Samples were diluted in the water bath of the dispersion system with a pump speed of 2,000 rpm (Hydro 2000MU), which is a laser diffraction based particle size analyzer [7, 29]. The relative refractive index (refractive index of sunflower oil/refractive index of water) of the different emulsions was 1.10. Samples were diluted in water in the dispersion. This determination was carried out in triplicate for each case.

## Statistical Analysis

Data were evaluated by analysis of variance (ANOVA) using the software Systat® 12.0 [30]. For this purpose, differences were considered significant at $p < 0.05$.

## Results and Discussion

### Compositional Analysis of Modified Lecithins

Native sunflower lecithin used as starting material presented the following composition: 43.1% phospholipids (16.5% PI, 16.2% PC, 5.3% PE, and 5.1% minor phospholipids), 33.4% oil, and 23.5% of other compounds (glycolipids, complex carbohydrates).

The phospholipid composition of different modified sunflower lecithins obtained in this work is shown in Table 1. A marked difference was recorded regarding the phospholipid composition between these samples and native sunflower lecithin. PC (PCF) and PI (PIF) enriched fractions exhibited the highest concentration of phosphatidylcholine (44.3%) and phosphatidylinositol (35.2%),

respectively. These results are in agreement with those obtained by applying different fractionation processes with absolute ethanol on soybean, rapeseed and sunflower lecithins [13, 14, 18]. Both hydrolyzed lecithins presented a high concentration of major lysophospholipids (>28.8%) compared to the native sunflower lecithin (≈1.1%), showing the efficiency of the enzymatic hydrolysis processes. In particular, the pancreatic $PLA_2$ (SHLP) produced a higher hydrolysis degree of the main phospholipids in comparison with the microbial phospholipase (SHLM).

## Optical Characterization of O/W Emulsions

Stability of the different O/W emulsions (30:70 wt/wt) was studied recording the backscattering (BS) profiles as a function of the cell length and time, by a vertical scan analyzer (QuickScan). For instance, Fig. 1 shows three typical profiles obtained for emulsions with addition of 0.1% of deoiled (DSL), PC enriched fraction (PCF) and a hydrolyzed sunflower lecithins (SHLM).

The creaming destabilization process (i.e., migration of oil particles to the upper portion of the tube) is evidenced by a decrease in %BS values at the bottom of the tube. The QuickScan profiles corresponding to the Zone I (10–20 mm) showed an increase of the emulsion stability against the creaming process, as a function of increasing concentration of different modified lecithins (Fig. 2). In particular, the addition of PCF and both hydrolyzed lecithins (SHLP and SHLM) generated a higher stability in O/W emulsions than DSL, over the studied range of concentration. In this sense, it should be noted that QuickScan profiles did not show significant variations of %BS values for 2.0% PCF, especially during the first 60 min. However, O/W emulsions with 0.1–0.5% of DSL and PI fraction showed a sharp decrease of %BS in the Zone I. These results are in concordance with those previously reported regarding the emulsifying activity of modified soybean lecithins [6].

The tube zone between 40 and 45 mm (Zone II) is characterized by the accumulation of oil droplets after the creaming process (cream phase); Fig. 3 shows the %BS values versus time in Zone II. Emulsions formulated with hydrolyzed lecithins presented higher %BS values than those obtained using the other modified lecithins, for all concentrations studied. The higher levels of %BS and the greater stability of these emulsions would be associated with the formation of dense cream phases with a lower proportion of continuous phase inside [31].

In addition, the use of the PC enriched fraction generated lower %BS values than those previously discussed for hydrolyzed lecithins, although the emulsions also produced a stable cream phase. However, emulsions with 0.1% of DSL and PIF did not allow the formation of the cream

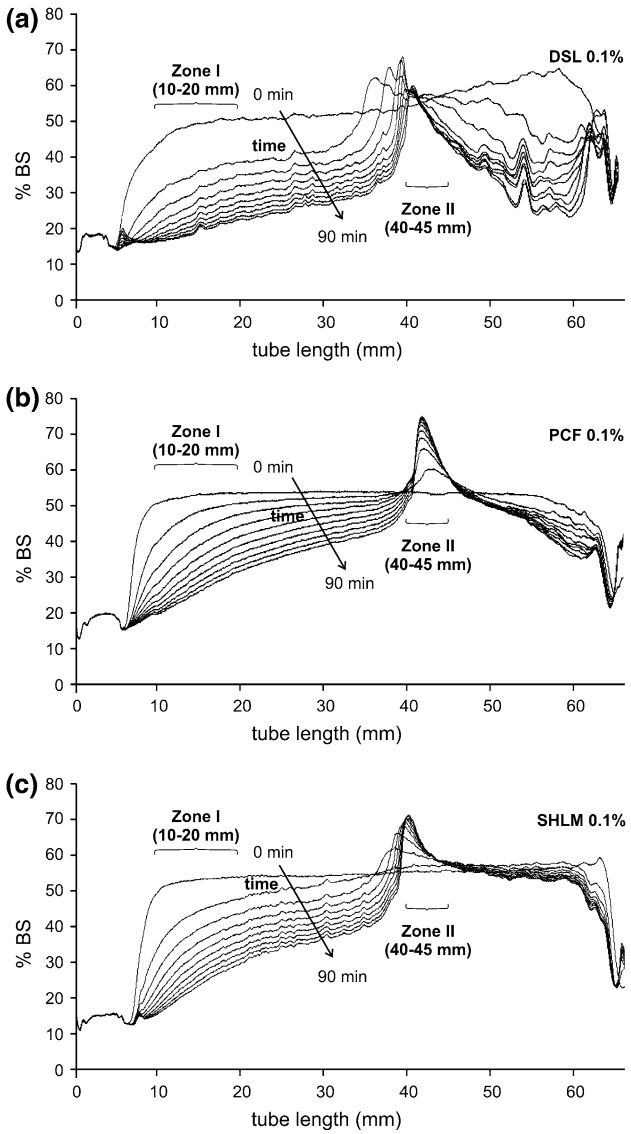


**Fig. 1** Backscattering (%BS) profiles of O/W emulsions (30:70 wt/wt) with the addition of **a** DSL 0.1%, **b** PCF 0.1% **c** SHLM 0.1%. For abbreviations see Table 1

phase. These results are related to the rapid decrease of %BS and the formation of an oil layer in the upper part of the tube (Fig. 1a) suggesting the occurrence of a cream phase destabilization, such as coalescence [23].

## Particle Size Distribution

Particle size distribution of O/W emulsions obtained with different modified lecithins was measured just after emulsification ($t = 0$), as an example the distribution in volume can be seen in Fig. 4. These distributions presented a bimodal or trimodal character depending on the concentration of aggregated lecithin, and the following particle size populations: (I) particle size <4 μm, (II) particle size

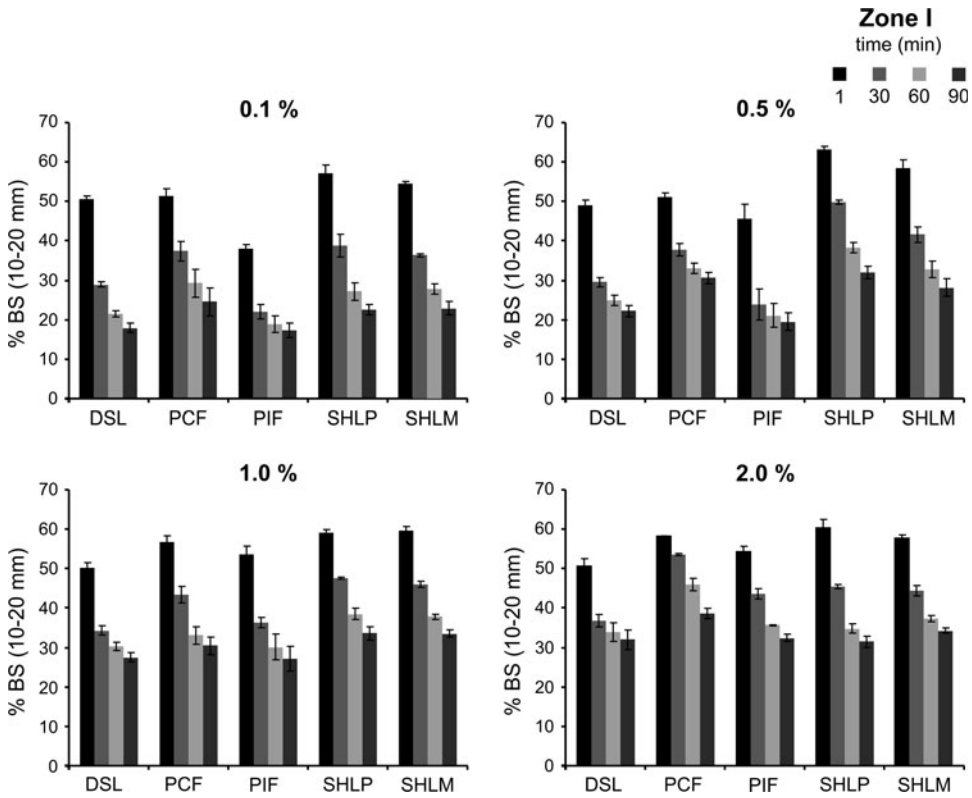


**Fig. 2** Backscattering (%BS) values of O/W emulsions (30:70 wt/wt) with the addition of different modified sunflower lecithins in Zone I (10–20 mm). Mean values ($n = 3$) ± SD

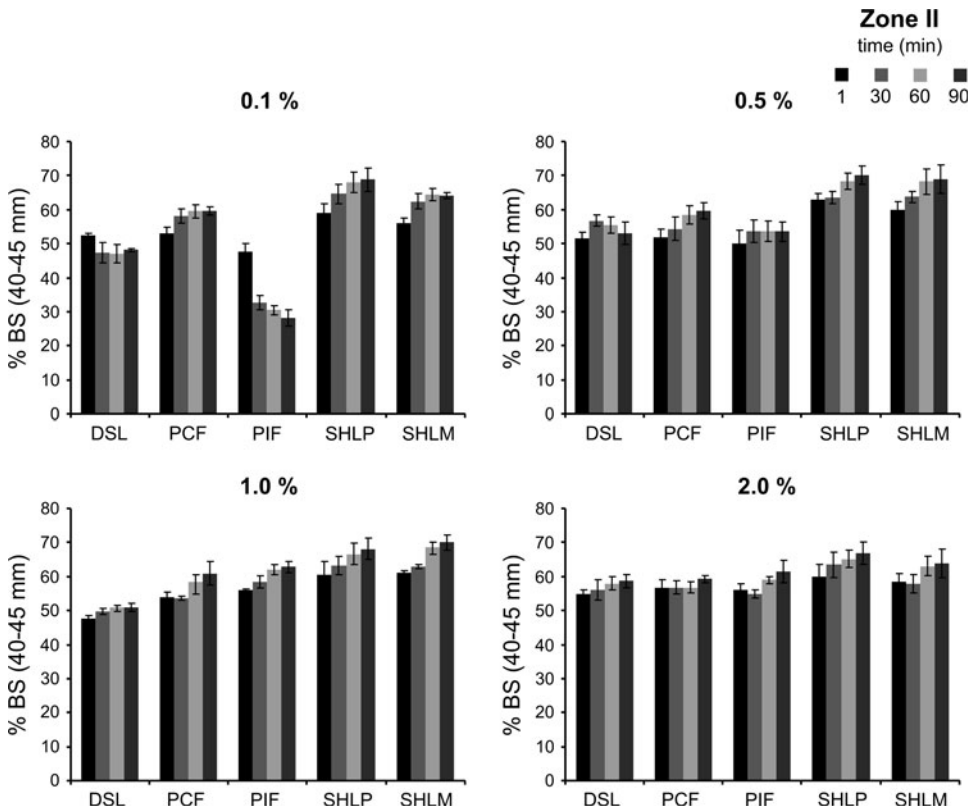


**Fig. 3** Backscattering (%BS) values of O/W emulsions (30:70 wt/wt) with the addition of different modified sunflower lecithins in Zone II (40–45 mm). Mean values ($n = 3$) ± SD

between 4 μm and 30 μm, (III) particle size >30 μm. In this sense, only PC enriched fraction (PCF) and hydrolyzed lecithin with the porcine pancreatic $PLA_2$ (SHLP) showed a trimodal character for all concentrations assayed. It should be noted that the SHLM presented a bimodal character for concentration in the range 0.1–1.0%, but with

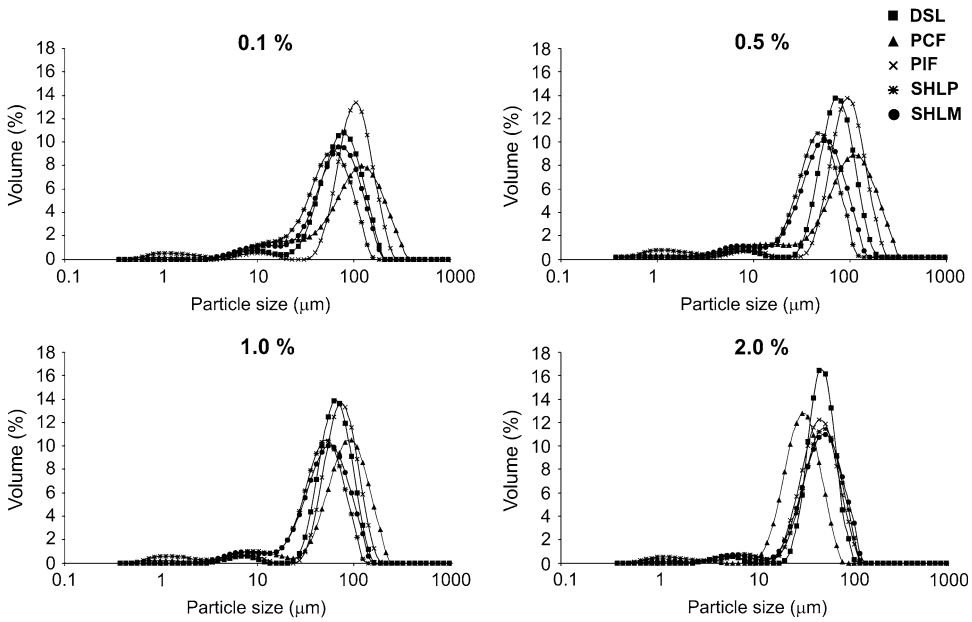


**Fig. 4** Volume particle size distribution for O/W emulsions with the addition of different modified sunflower lecithins. Mean values ($n = 3$)

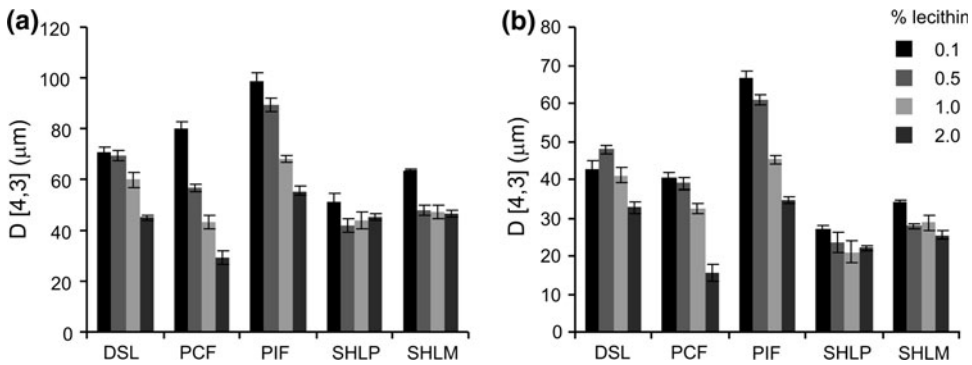


**Fig. 5** De Brouckere (D [4, 3]) and Sauter (D [3, 2]) mean diameters for O/W emulsions with the addition of different modified sunflower lecithins. Mean values ($n = 3$) ± SD

a high percentage of particles of the population II in comparison with DSL and PIF.

In order to complete the analysis of particle size distribution, Fig. 5 depicts the evolution of De Brouckere (D [4, 3]) and Sauter (D [3, 2]) mean diameters as a function of the concentration of different emulsifiers. Modified sunflower lecithins enhanced their potential emulsifying activity as their concentration was increased. Both hydrolyzed lecithins generated values of D [4, 3] and D [3, 2] significantly lower than other modified lecithins, at low concentration (0.1–0.5%). However, at levels of 2%, PC enriched fraction (PCF) presented the lowest mean diameters. These results are correlated with the high stability of the O/W emulsions recorded when using PC fraction and hydrolyzed lecithins, considering the main destabilization processes determined by the corresponding QuickScan profiles (creaming or coalescence). It is worth noting that a high concentration of small particles produces a slow creaming process, according to the Stokes' law [7, 31].

The hydrophilic-lipophilic balance value (HLB) is often used in connection with the performance of emulsifiers [7]. The higher concentration of hydrophilic phospholipids (PC, lysophospholipids) presented in the PC enriched fraction (PCF) and both hydrolyzed lecithins (SHLP and SHLM) increase this empirical value. In this sense, according to Carlsson [32], these modified lecithins with higher HLB values presented the best properties as O/W emulsifying agents.

Also, the phase structure at the interface of the different phospholipids influences the emulsion formation and stability [6]. PC forms a lamellar phase at the interface between oil and water with well ordered mono- and bilayers, and LPC and LPE form hexagonal wide spread clusters. These structures have a great importance for the stabilization of O/W emulsions. This behaviour is in relation to the low mean diameters and the high concentration of small particle populations registered in emulsions using PCF and both hydrolyzed lecithins (Figs. 4, 5). However, PE gives reversed hexagonal phase, which are more difficult to arrange at the interface [33]. The presence of PE could explain the poor characteristics as emulsifying agent of DSL and PIF at low concentration, and the higher mean

diameters when was using SHLM in contrast to when using SHLP (Fig. 5).

Taking into account the results presented in Figs. 2, 3, 4, 5, the addition of a concentration between 0.5 and 1.0% of hydrolyzed lecithin (SHLP, SHLM) is sufficient for covering all the droplet surface. Higher concentrations of this modified lecithin do not show significant differences in the % BS values, nor in the mean particle sizes. However, DSL, PIF and PCF presented an improvement in the stability of O/W emulsions as a function of increasing concentration. In this sense, PCF showed the best characteristics as emulsifying agent for addition of 2%.

The use of a microbial phospholipase $A_2$ gives the possibility of obtaining diverse sunflower lysolecithins, whose functionality could be applied to the development of foods with *kosher* and *halal* certification.

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