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Characterization and emulsifying properties of different sunflower phosphatidylcholine enriched fractions

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Running title: Characterization and emulsifying properties of PC fractions

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Abbreviations: BS: Back scattering; E_{PL}: extraction coefficient; O/W: oil in water; PA: phosphatidic acid; PC: phosphatidyl choline; PE: phosphatidyl ethanolamine; PI: phosphatidyl inositol; PLs: phospholipids

Summary

Fractions enriched in specific phospholipids are desirable for different industrial purposes because of their characteristic physicochemical and functional properties. We studied the fractionation process of sunflower lecithin using different absolute-ethanol:water ratios and pHs and then evaluated the emulsifying properties of phosphatidyl-choline- (PC-) enriched fractions in oil:water 30:70 (v/v) mixtures. We observed a high recovery of PC and a low content of phosphatidyl inositol in all the PC-enriched fractions thus obtained along with the highest yields of this phospholipid after extraction with an absolute-ethanol:water mixture of 96:4 (v/v). The use of different pH values for the different solvent extraction media did not markedly modify the yield of the enriched fractions. The extraction coefficients for PC and phosphatidyl ethanolamine (PE) evidenced an increase in both these phospholipids in the PC-enriched fractions upon extraction with the higher concentration of absolute ethanol. Emulsions containing the PC-enriched fractions obtained with the absolute-ethanol:water mixture of 96:4 (v/v) exhibited the highest stability at pH 7.5 because of the high PC/PE ratio compared to that of the PC-enriched fractions extracted with the absolute-ethanol:water mixture of 90:10 (v/v). A high emulsifier concentration resulted in a low mean D-[4,3] diameter of the particles and a high stability.

Practical applications: Sunflower lecithin is a promising alternative to soybean lecithin because it is considered the product of a non–genetically modified organism (non-GMO). Experimentation on lecithin modification under industrial conditions with adequate techniques of analysis may be useful for evaluating the potential applications of these sunflower by-products to the production of new emulsifiers. Thus, an analysis of the influence of different fractionation parameters, such as the pH of the solvent extraction, on the composition and emulsifying properties of the resulting PC-enriched fractions may provide useful information for the food industry.

1 Introduction

Sunflower lecithin is obtained as a by-product of the oil-refining process in order to enhance physical oil stability [1]. Lecithin is a mixture of acetone-insoluble phospholipids, containing mainly phosphatidyl choline (PC), phosphatidyl ethanolamine (PE), phosphatidyl inositol (PI), minor compounds such as phosphatidic acid (PA), and other substances (*e. g.*, triglycerides, carbohydrates). 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 [2].

Phospholipids exhibit a differential solubility in ethanol, with PC being preferentially soluble but PE distributed more or less evenly between the soluble and the insoluble phases. The most acidic phospholipids such as PI and PA remain practically insoluble after solvent extraction and are mainly recovered in the extraction residue. These partitioning properties have been employed in fractionations aimed at to modifying the phospholipid composition of different native lecithins, improving their properties, or meeting particular functional requirements [3]. Cabezas *et al.* [2] studied different operating conditions for the ethanolic fractionation of sunflower lecithin and recorded the influence of temperature, extraction time, solvent-lecithin ratio, and absolute-ethanol concentration on the degree of enrichment in the soluble and insoluble fractions obtained, but the effect of pH of the solvent extraction has still not been investigated in this type of lecithin.

The phospholipid distribution of each enriched fraction has been shown to be quite strongly dependent on the pH of the extraction solvent. Moreover, the solubility of the phosphatides is affected differently: PC and PE are zwitterionic at neutral pH because of the presence of the amino group in the molecule, whereas PI has a net negative charge (is anionic) at this pH [4, 5]. The various head groups of phospholipids furthermore give them different polarities and emulsification properties [6] that are, in turn, directly associated with the net charge of the molecules [7].

Emulsions are unstable systems physicochemically, passing from an initially homogeneous suspension to a separation into two phases upon standing. The surface-activity properties of an emulsifier depend on its chemical structure. Different types of instability common to all dispersed systems, such as creaming, flocculation, and coalescence of oil droplets can be decreased through the use of emulsifiers [8, 9].

PC-enriched soluble fractions obtained by ethanol fractionation of vegetable lecithin are believed to be good oil-water (O/W) emulsifiers as a result of the high PC/PE ratio and the lamellar structure of the PC at the oil-water interface [10].

The aim of this work was to study the effect of the use of different absolute-ethanol:water mixtures and pHs on the differential extraction of phospholipids from sunflower lecithin and to evaluate the emulsifying properties in O/W emulsions of the different PC-enriched fractions thus obtained. On the basis of these considerations, the present study was undertaken with the objective of providing the food industry with useful information for the development of the appropriate surface-active emulsifiers for use in specific manufacturing contexts.

2 Materials and Methods

2.1 Materials

The native sunflower lecithin used as starting material was provided by a local oil industry (Vicentin S.A.I.C. - Rosario, Argentina). All solvents used were of analytical grade.

2.2 Sunflower lecithin fractionation

A fractionation of sunflower lecithin was carried out according to Cabezas *et al.* [2] in order to obtain a high PC concentration in the soluble phase. The initial extraction, carried out on 30 g of crude sunflower lecithin, involved the addition of two absolute-ethanol:water mixtures, 96:4 or 90:10 (v/v), at three different pHs (3.3, 7.5, and 10.0), adjusted with hydrochloric acid or ammonia, at an absolute-ethanol:lecithin ratio of 3:1 (v/w). Each sample was incubated in a water bath at 65 °C for 90 min with moderate agitation and then centrifuged at 1,880 *g*. Upon separation of the corresponding ethanolic extracts and the solid residues under each condition, the ethanol was eliminated by evaporation under vacuum.

The aqueous-ethanol–soluble and –insoluble phases were further deoiled with acetone, according to the Official Method Ja 4-46, procedures 1-5, of the American Oil Chemists' Society [11], to obtain PC- and PI-enriched fractions, respectively. Both fractions were then stored at 0 ºC. This fractionation procedure was performed in duplicate.

The yield associated with each fraction was calculated according to the equation:

$$\text{Enriched Fraction Yield}\,(\%) = \frac{\text{amount of fractionated sunflower lecithin}}{\text{amount of starting sunflower lecithin}} \cdot 100$$

The following equation must be considered:

PC-enriched–fraction yield (%) + PI-enriched–fraction yield (%) + % oil = 100%

2.2.1 Phospholipid composition

The phospholipid composition of the PC- and PI-enriched fractions 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) [12]. For this purpose, 100 mg of each of the two fractions under each condition were 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 spectroscopic technique. 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).

Phospholipid composition of PC and PI enriched fractions obtained under different conditions of the fractionation process was determined by ^{31}P NMR analysis in a Bruker Avance 600 MHz automatic spectrometer, using triphenyl phosphate as internal standard (Spectral Service GmbH,

Köln, Germany) [12]. For this purpose, 100 mg of each modified lecithin were 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 shaking, and analyzed by the described spectroscopic technique. Phospholipid composition was expressed in terms of molar concentration (mol / 100 mol lecithin), thus the phospholipid content (% PC, % PI and % PE) of each fraction was obtained.

2.2.2 Extraction coefficients

We monitored the differential fractionation of each phospholipid by the two absolute-ethanol/water mixtures and calculated the corresponding extraction coefficients $\%E_{PL}$ (% E_{PC}, % E_{PE} and $\%E_{PI}$) for both types of enriched fractions. The resulting values represent the percent contribution of each phospholipid in these fractions [13] according to the following equation:

$$\%E_{PL}\ (\text{PC enriched fraction}) = \frac{m_{PL}\ (\text{PC enriched fraction})}{m_{PL}\ (\text{PC enriched fraction}) + m_{PL}\ (\text{PI enriched fraction})} . 100$$

where

PL: PC, PE or PI

m_{PL} (PC enriched fraction) = PC enriched fraction yield % * % PL (PC enriched fraction)

m_{PL} (PI enriched fraction) = PI enriched fraction yield % * % PL (PI enriched fraction)

The following expression must be considered for calculations:

$$\%E_{PL}\ (\text{PC enriched fraction}) + \%E_{PL}\ (\text{PI enriched fraction}) = 100\%$$

Mathematical models were obtained according to different operating variables of the sunflower-lecithin fractionation by means of the SYSTAT 12® software [14].

The experimental design used in the calculations was based on a full factorial design. The data were evaluated by analysis of variance (ANOVA). Significant variables and their interactions (p <0.05) were used to fit the experimental data in the following second-order polynomial equation and then obtain the corresponding coefficients:

$$Y = \beta_o + \sum_{i=1}^{4} \beta_i x_i + \sum_{i=1}^{3} \sum_{j=i+1}^{4} \beta_{ij} x_i x_j$$

where Y is the response variable (E_{PC} in PC-enriched fraction), X_i and X_j are the coded independent variables, and β_o, β_i, β_{ij} are the regression coefficients of variables for the intercept, linear and interaction regression terms, respectively [17].

2.3 O/W emulsions

O/W emulsions (30:70 [w/w]) were prepared by the addition of the different PC-enriched fractions at between 0.1% and 2.0% (w/w) with respect to the aqueous phase at room temperature followed by homogenization in an ULTRA-TURRAX T25 homogenizer for 1 min at 10,000 rpm [15].

2.3.1 Emulsion stability

The physical stability of the O/W emulsions (30:70, [w/w]) immediately after homogenization were followed by measuring the variation in the percentage of backscattering (% BS) from the 850-nm near-infrared beam of a Vertical Scan Analyzer (QuickScan, Beckman Coulter, Fullerton, CA, USA) along the height of the sample (65 mm) every min for 60 min [16]. The stability of the different O/W emulsions was monitored by recording the differential-scanning profiles of the informative zones at the bottom (zone I at 10–15 mm) and the top (zone II at 50–55 mm) of the tube as a function of time.

2.3.2 Droplet-size measurements

Aliquots of the emulsions were monitored with a particle-size analyzer (Malvern Mastersizer 2000E, Malvern Instruments Ltd., Worcestershire, U.K.). De-Broucker (D [4,3]) mean diameters were determined immediately after homogenization. Samples were diluted in the water bath of the dispersion system having a pump speed of 2,000 rpm (Hydro 2000MU). The relative refractive index (refractive index of sunflower oil/refractive index of water) of the different emulsions was 1.10 [15]. This determination was carried out in duplicate for each sample.

2.4 Statistical analysis

Data were evaluated by analysis of variance (ANOVA) through the use of the SYSTAT 12® software. For this purpose, differences were considered significant at a $p < 0.05$.

3 Results and Discussion

3.1 Sunflower lecithin

The sunflower lecithin used as the starting material had the following phospholipid composition: PC 16.4%, PI 17.1%, PE 6.3%, PA 2.0%, other phospholipids 0.7%.

3.2 Enriched-fraction yield (%)

Table 1 shows the percent yields for the sunflower PC- and PI-enriched fractions obtained by fractionation with the absolute-ethanol:water mixtures (96:4 and 90:10 [v/v]) at different pHs (3.3,

7.5, and 10.0). The PC-enriched fraction yield increased from 12.5% (90:10, pH 3.3) to 15.6% (96:4 pH 3.3) with the higher proportion of absolute-ethanol in the solvent extraction, whereas the PI fraction decreased from 51.9% (90:10, pH 3.3) to 48.3% (96:4, pH 10.0). This opposite yield on the part of the two PL fractions could have resulted because PLs exhibit poor solubilities in water and aqueous solutions [3, 5]; and therefore with the higher ethanol content the greater yield of total PLs in the PC-enriched fraction left behind a lower PL content in the residue from which the PI enriched fraction was extracted. These results evidence the efficiency of the fractionation achieved in this work. No significant differences (p >0.05), however, were detected for the two different fraction yields as a function of the pH for each of the absolute-ethanol:water mixtures assayed.

3.3 Phospholipid composition (^{31}P NMR)

Table 2 gives the phospholipid composition of the different PC- and PI-enriched fractions. The PC-enriched fractions obtained after the different solvent extractions contained high levels of PC but only low amounts of PI. This enriched fraction from the absolute-ethanol:water 90:10 (v/v) extracts exhibited a greatly enhanced concentration of PC (≥45), but the percentage of PI was also significantly increased (p <0.05) compared to the concentration in the fraction extracted with absolute-ethanol:water 96:4 (v/v).

In contrast, the PI-enriched fractions exhibited significant differences (p <0.05) in phospholipid composition, with the proportion of PI increasing as a function of absolute-ethanol concentration and that of PC and PE decreasing. These results are in agreement with those previously obtained with different concentrations of absolute-ethanol in the fractionation of sunflower lecithin [2, 17].

3.4 PC/PE ratio

Different types and concentrations of alcohol have been used to obtain specific extraction yields and PC/PE ratios [8]. Table 2 shows the PC/PE ratio for the PC- and PI-enriched fractions obtained as a function of the proportion and pH of different absolute-ethanol:water extraction mixtures. These ethanol-soluble fractions exhibited a high PC/PE ratio (≥7.0); the PC-enriched fraction obtained by fractionation at pH 7.5 with an absolute-ethanol:water mixture of 96:4 (v/v) resulted in the highest PC/PE ratio (8.6); whereas the fraction from the insoluble fraction had an extremely low PC/PE ratio (≤1.6). These results illustrate the marked differences in phospholipid composition between the enriched fractions and the native sunflower lecithin—it at a PC/PE ratio of 2.6.

3.5 Extraction coefficients (%E_{PL})

We determined the $\%E_{PL}$ extraction coefficients ($\%E_{PC}$, $\%E_{PI}$ and $\%E_{PE}$) under different processing conditions taking into account the percent yields and the phospholipid compositions of the PC- and PI-enriched fractions (Table 3). The efficiency of sunflower-lecithin fractionation in the present work was carried out by analyzing the $\%E_{PL}$ values based on the solubility in ethanol of the different PLs (*cf.* reference 17). For the PC-enriched fractions, the $\%E_{PC}$ and $\%E_{PE}$ values indicated a significant increase (p <0.05) in the solubility of both phospholipids (PC and PE) at the higher ethanol concentration in the extraction solvent. In contrast, the $\%E_{PI}$ was low (<4.0%) under all the conditions assayed, thus indicating a very low solubility of PI.

We applied mathematical models in order to explain the effect of the operating variables of the fractionation on the resulting $\%E_{PL}$ values in the PC-enriched fraction. These mathematical models were based on the regression of quadratic polynomial equations that included the overall incidence of the independent variables (the percentage of absolute-ethanol, the pH, and the interaction of the two) when significant (p <0.05) as judged by ANOVA. On the basis of the final models the following equations and also their respective averaged absolute relative errors (AARE) were calculated:

$\%E_{PC} = 49.823 + 4.922\ EtOH$ $\quad R^2 = 0.916$, AARE (%) = 2.29

$\%E_{PI} = 2.749 - 0.452\ EtOH + 0.158\ pH$ $\quad R^2 = 0.951$, AARE (%) = 13.14

$\%E_{PE} = 16.076 + 2.011\ EtOH - 0.777\ EtOH*pH$ $\quad R^2 = 0.951$, AARE (%) = 2.57

where variables were coded as +1 and -1 and EtOH = percentage of absolute-ethanol.

The influence of significant variables was associated with the percentage of explained variance. Thus, the percentage of absolute-ethanol was highly significant for all extraction coefficients ($\%E_{PC}$, $\%E_{PE}$ and $\%E_{PI}$) with recorded values of explained variance of 88.89, 76.30, and 87.68%, respectively. The pH was significant only in the extraction of PI, there representing 22.35% of the explained variance of $\%E_{PI}$, while EtOH*pH interaction was significant for $\%E_{PE}$, there representing 10.55% of the explained variance for $\%E_{PE}$.

The linear models obtained showed a good correlation with the experimental data for the extraction coefficients $\%E_{PC}$, $\%E_{PI}$ and $\%E_{PE}$. The AARE suggested an acceptable accuracy for the coefficients determined under the different operating conditions on a laboratory scale [17].

3.6 Emulsions: destabilization kinetics

The stability of the different O/W emulsions (30:70 [w/w]) containing 0.1% to 2.0% of the PC-enriched fractions was determined optically by recording the %-BS profiles along the length of the cell and over time. The destabilization of the O/W emulsions was followed by monitoring the

sequential profiles obtained in the bottom zone I (10–15 mm)—that area being where a clarification of the emulsion is produced by the migration of the emulsified droplets from the bottom toward the top of the tube (*i, e.*, creaming)—and the upper zone II (50–55 mm)—that portion being the location of the potential destabilization of the cream phase of the emulsions through coalescence [14].

Fig. 1 shows two typical profiles corresponding to O/W emulsions (30:70 [w/w]) containing (a) 2.0% (v/v) and (b) 0.5% (v/v) of the PC-enriched fractions extracted with absolute-ethanol and water at a ratio of 96:4 (v/v) and pHs of 7.5 and 10, respectively The %-BS values in zone I of Fig. 1a were reduced with time through a process of destabilization akin to creaming produced by the migration of oil droplets from the bottom of the tube to the top. In contrast, in Fig. 1b the %-BS values are seen to decrease with time along the length of the cell. This dynamic could be related to a creaming-type process followed by a coalescence resulting in a decrease in the number of droplets consequent to an increase in droplet size. A rapid separation of the creamed phase followed by a destabilization by coalescence was recorded at the top of the tube (zone II), with the creaming and coalescence both occurring simultaneously [9].

Fig. 2 illustrates the destabilization kinetics of O/W emulsions containing four different percent concentrations of PC-enriched fractions that had been extracted with an absolute-ethanol:water mixture of 90:10 (v/v) at three different pHs. The destabilization is evidenced by a rapid decrease in the %-BS values at the bottom of the tube at all four emulsifier concentrations and the three pHs (Fig. 2a). This pattern results from the ascent of oil particles to the uppermost part of the tube. In Fig. 2b the destabilization of the cream phase through coalescence occurs at concentrations of 0.1% to 0.5% (v/v) of the emulsifying agent, resulting in a decrease in the %-BS values. When the concentration of the emulsifier was at 1.0% to 2.0% (v/v), however; a stable, coalescence-resistant cream phase was formed at all pHs (Fig. 2b). The lowest cream-phase stability was observed at pH 3.3 with these PC-enriched fractions.

Fig. 3 shows the destabilization kinetics of O/W emulsions containing the four different percent concentrations of PC-enriched fractions, but that had been extracted with an absolute-ethanol:water mixture of 96:4 (v/v) at the same three pHs. The QuickScan™ profiles corresponding to zone I (Fig. 3a) showed an increase in the %-BS values after the addition of the PC-enriched fraction obtained at pH 7.5 of the solvent extraction, with high emulsion stability against the creaming process for all concentrations assayed. In contrast, the PC-enriched fractions obtained at pH 3.3 and 10.0 at this higher ethanol:water ratio showed a low emulsifying capacity under all the conditions studied. Furthermore, in the uppermost part of the tube (zone II, Fig. 3b) a coalescence destabilization of the cream phase occurred at low concentrations (0.1% and 0.5% [v/v]) of emulsifier, with a similar

result obtaining in emulsions with the PC-enriched fractions extracted at an absolute ethanol:water ratio of 90:10 (v/v), as was shown in Fig. 2b. At the increased concentrations of 1.0% and 2.0% of the emulsifying agent, however, the emulsions with the PC-enriched fractions extracted at pH 3.3 and 7.5 formed a stable cream phase: with the emulsifiers from both the ethanol-water extracts, the upper zone II showed higher levels of % BS at both of those concentrations of PC-enriched fractions. This stability is caused by the formation of a cream phase with a lower proportion of continuous phase that, as a result of Stokes's law, produces a slow creaming process [18].

The more pronounced emulsification ability of the PC-enriched fraction from the higher ethanol-containing extracts at pH 7.5 as demonstrated in Fig. 3a could reflect the high PC/PE ratio of that fraction (*cf.* Table 2). The structure of the different phospholipids at an oil-water interface influences emulsion formation and stability [10]. PC forms a lamellar structure at such an interface with well ordered monolayers and bilayers; whereas PE assumes a reverse-hexagonal arrangement at the interface that is more difficult to attain [19]. Because of the high PC/PE ratio and consequent interface arrangement of the PC-enriched fraction obtained through lecithin fractionation at pH 7.5 with an ethanol:water ratio of 96:4, that preparation could thus be considered a good oil-water (O/W) emulsifier.

3.7 Droplet size

Table 4 shows the mean diameters of the droplets in D [4,3] values in the different emulsions. According to Relkin and Sourdet [20], D [4,3] is a sensitive parameter for analyzing oil-droplet aggregation (through coalescence and/or flocculation).

The emulsion containing 0.1% (v/v) of the PC-enriched fraction tended to coalesce quickly because the amount of emulsifying agent was not enough to completely cover the surface of the oil droplets (data not shown). The mean diameter D [4,3] decreased progressively and significantly (p <0.05) with increased concentrations of the PC-enriched fractions above 0.5% (v/v), so that the concentration of 2.0% (v/v) resulted in the lowest droplet diameters at all three pHs in emulsions made with both absolute-ethanol:water extracts. These QuickScan-profile results correlated with the enhanced stability of the O/W emulsions at increased concentrations of the PC-enriched fractions.

Emulsions with the higher percentages of the PC-enriched fractions (>0.5% [v/v]) did not exhibit significant differences (p >0.05) in stability whether those extracts had been performed at either of the absolute-ethanol:water ratios or at any of the three pHs. Nevertheless, the highest observed stability of the emulsions—it occurring with the PC-enriched fraction extracted with the absolute-ethanol:water ratio of 96:4 at pH 7.5—could be related to the highest proportion of PC/PE present

in that extract, which differential phospholipid content provided a high degree of stability of the oil droplets over time.

4 Conclusions

The work presented here investigated the fractionation of sunflower lecithin at two absolute-ethanol:water ratios and three pHs. The higher ethanol concentration in the extraction resulted in an enhanced yield of PC in the extract obtained at any of the three pHs.

The PC-enriched fraction extracted at absolute-ethanol:water ratio of 96:4 and a pH of 7.5 contained the highest PC/PE ratio, which relative proportion of the two phospholipids—through their interaction with water and their geometry of assembly at the oil-water interface—was found to contain the optimal properties conducive to emulsification.

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The authors declare no conflict of interest.

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Figure 1. Backscattering (%-BS) profiles of oil-water emulsions (30:70 [w/w]) containing the indicated percent of PC-enriched fraction obtained by extraction with an absolute-ethanol:water mixture of 96:4 (v/v): **a)** 2.0% at pH 7.5 **b)** 0.5% at pH 10.0

Figure 2. Backscattering (% BS) vs. time of oil-water emulsions (30:70 [w/w]) containing the PC-enriched fraction obtained by extraction with an absolute-ethanol:water mixture of 90:10 (v/v) at concentrations of 0.1%, 0.5%, 1%, or 2%. **(a)** Zone I (10–15 mm), **(b)** zone II (50–55 mm). Black bars, pH 3.3; dark-gray bars, pH 7.5, light-gray bars, pH 10.0; mean values ± SD (n = 2)

Figure 3. Backscattering (% BS) vs. time of oil-water emulsions (30:70 [w/w]) containing the PC-enriched fraction obtained by extraction with an absolute-ethanol:water mixture of 96:4 (v/v) at concentrations of 0.1%, 0.5%, 1%, or 2%. **(a)** Zone I (10–15 mm), **(b)** zone II (50–55 mm). Black bars, pH 3.3; dark-gray bars, pH 7.5, light-gray bars, pH 10.0; mean values ± SD (n = 2)

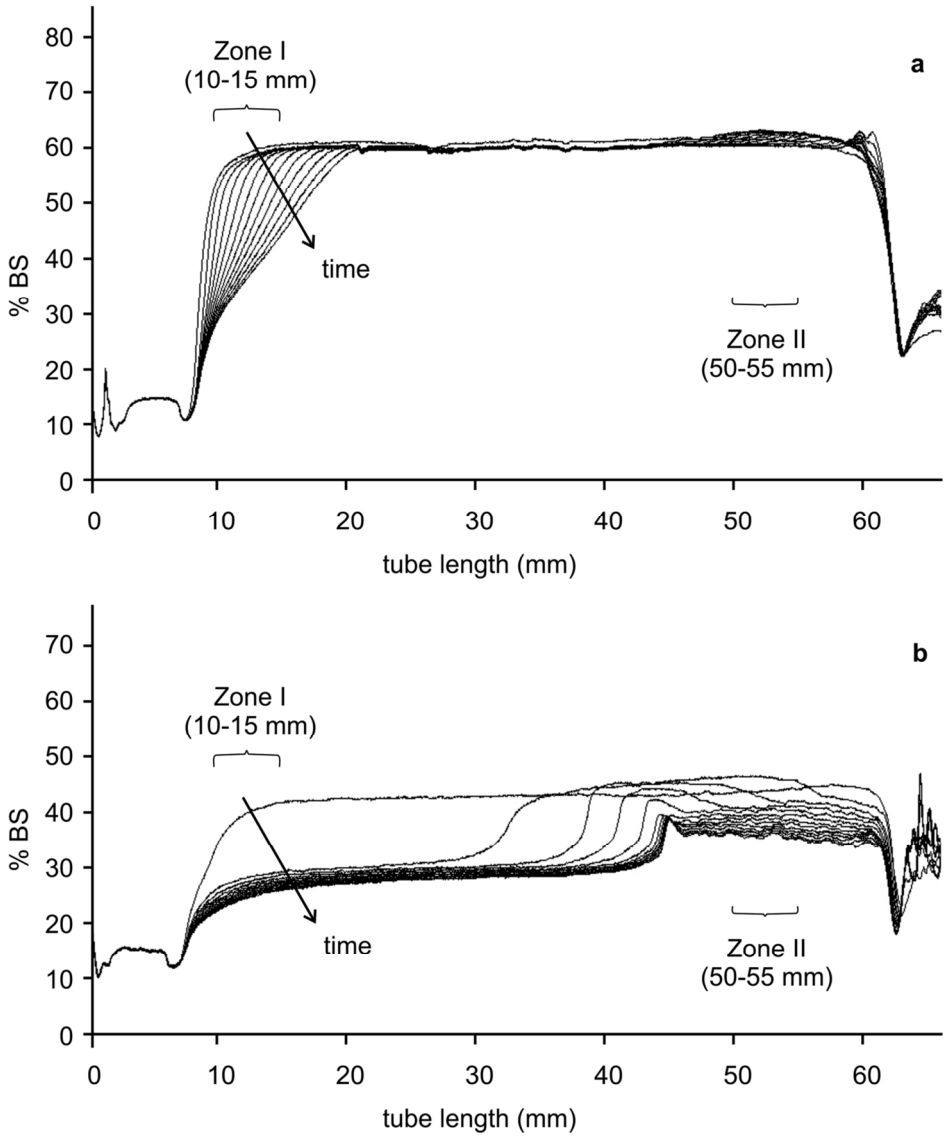


Figure 1, Guiotto et al.
Backscattering (% BS) profiles of O/W emulsions (30:70 wt/wt) with the addition of PC enriched fractions obtained by fractionation with ethanol:water mixtures 96:4 (vol/vol) a) 2.0% pH 7.5 b) 0.5% pH 10.0
122x149mm (300 x 300 DPI)

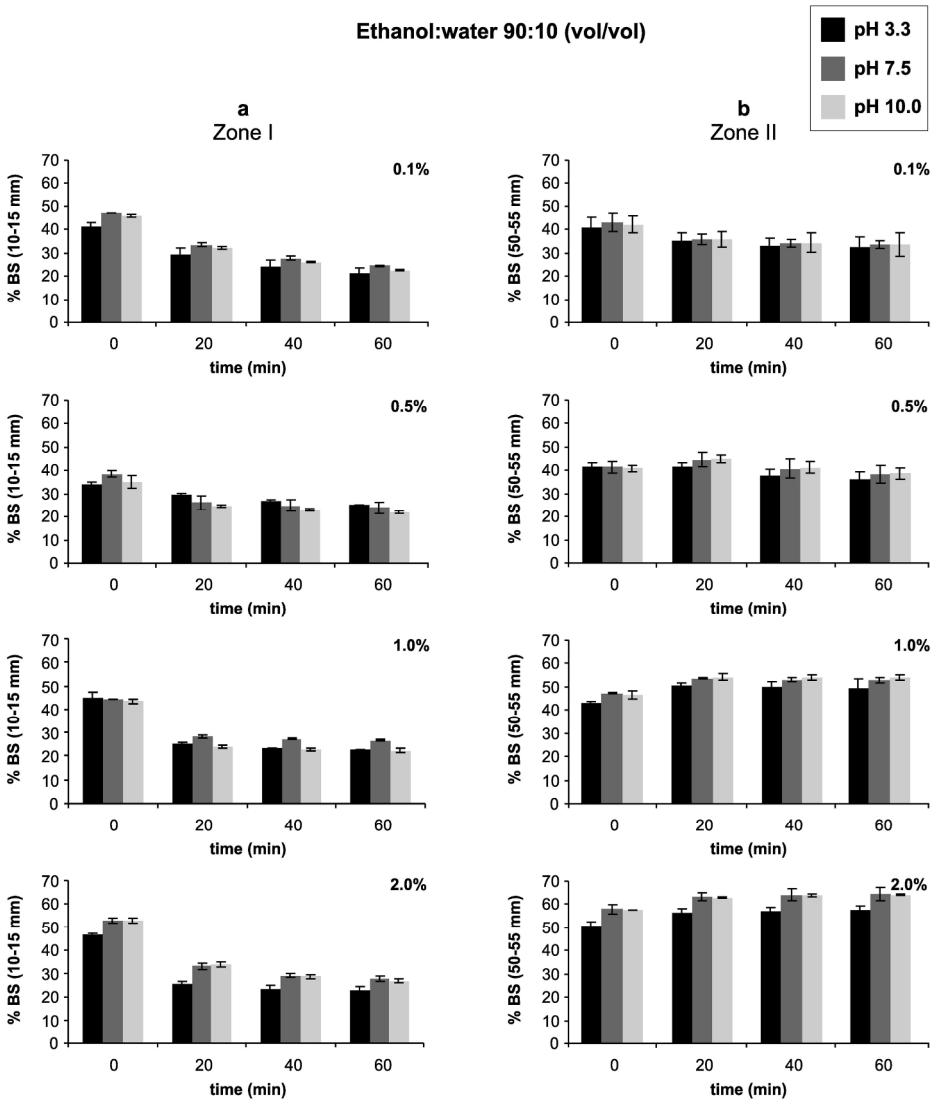


Figure 2, Guiotto et al.
Backscattering (% BS) vs. time of oil-water emulsions (30:70 [w/w]) containing the PC-enriched fraction obtained by extraction with an absolute-ethanol:water mixture of 90:10 (v/v) at concentrations of 0.1%, 0.5%, 1%, or 2%. (a) Zone I (10–15 mm), (b) zone II (50–55 mm). Black bars, pH 3.3; dark-gray bars, pH 7.5, light-gray bars, pH 10.0; mean values ± SD (n = 2)
226x259mm (300 x 300 DPI)

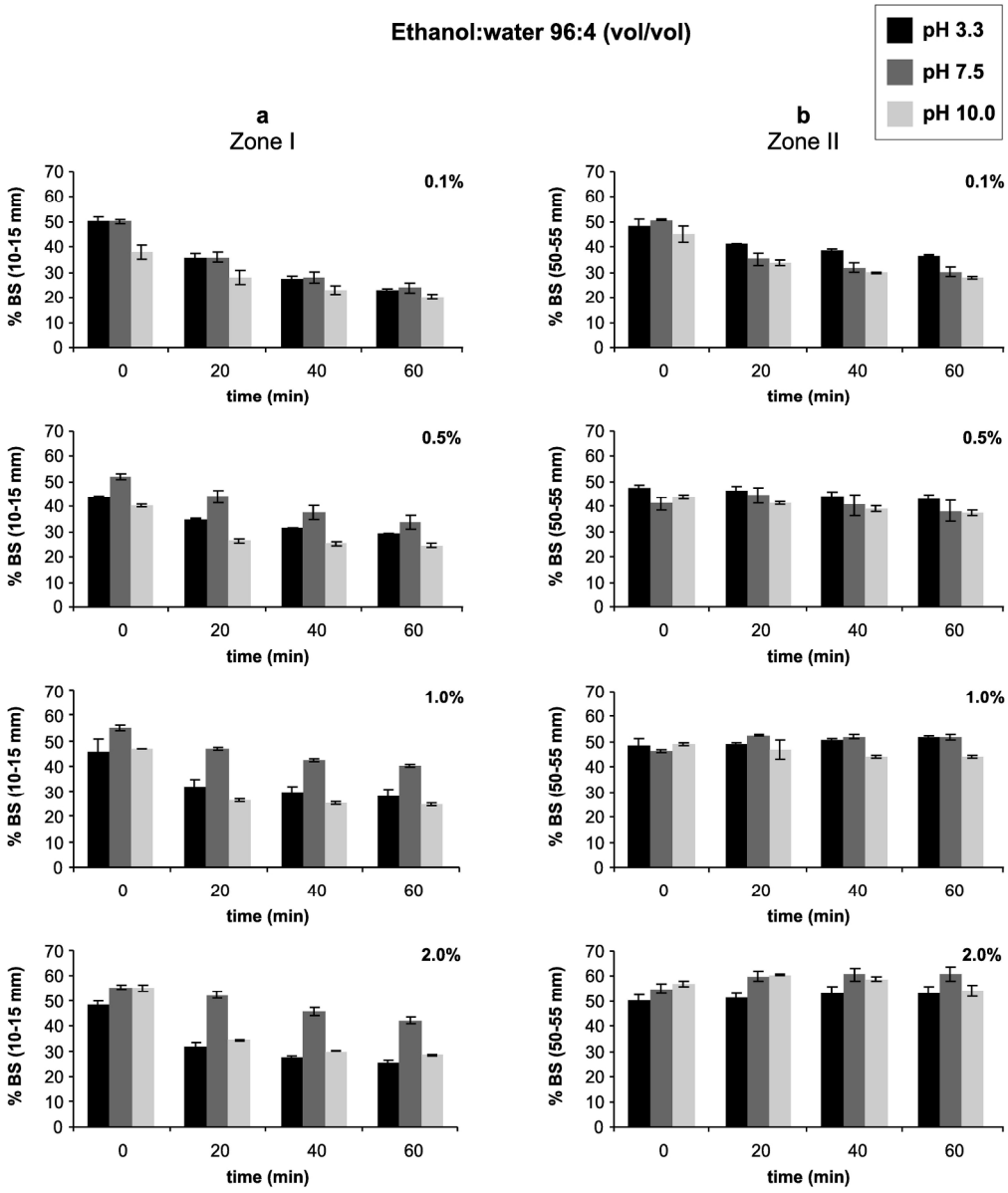


Figure 3, Guiotto et al.
Backscattering (% BS) vs. time of oil-water emulsions (30:70 [w/w]) containing the PC-enriched fraction obtained by extraction with an absolute-ethanol:water mixture of 96:4 (v/v) at concentrations of 0.1%, 0.5%, 1%, or 2%. (a) Zone I (10–15 mm), (b) zone II (50–55 mm). Black bars, pH 3.3; dark-gray bars, pH 7.5, light-gray bars, pH 10.0; mean values ± SD (n = 2)
216x257mm (300 x 300 DPI)

Table 1. Yield of PC- and PI-enriched fractions (%) obtained by extraction of sunflower lecithin as a function of the composition and pH of the partitioning solvent

Ethanol:water [v/v]	pH	Yield of PC-enriched fraction [%]	Yield of PI-fraction fraction [%]
90:10	3.3	12.5^{a}	51.9^{d}
	7.5	12.7^{ab}	51.5^{cd}
	10.0	13.8^{b}	50.9^{c}
96:4	3.3	15.6^{c}	49.0^{ab}
	7.5	15.5^{c}	49.3^{b}
	10.0	15.2^{c}	48.3^{a}

Average values (n = 2). Values within a column with different superscript letters are significantly different (p <0.05); those with two superscripts are not significantly different from the figures with either of the same single superscripts.

Table 2. Phospholipid composition of PC- and PI-enriched fractions (percent molar concentration relative to the total concentration of the fraction) determined by ^{31}P NMR

		PC-enriched fraction				PI-enriched fraction			
Ethanol:water [v/v]	**pH**	**%PC**	**%PI**	**%PE**	**PC/PE**	**%PC**	**%PI**	**%PE**	**PC/PE**
90:10	3.3	46.2^{a}	4.1^{c}	6.2^{b}	7.4^{ab}	14.7^{c}	30.4^{a}	9.4^{b}	1.6^{c}
	7.5	46.0^{a}	3.8^{c}	6.2^{b}	7.4^{ab}	14.2^{bc}	30.0^{a}	9.6^{b}	1.5^{bc}
	10.0	46.0^{a}	4.0^{c}	6.1^{b}	7.5^{ab}	13.5^{b}	30.2^{a}	9.5^{b}	1.4^{b}
96:4	3.3	44.8^{a}	2.6^{b}	5.6^{a}	7.9^{b}	11.6^{a}	33.0^{bc}	7.6^{a}	1.5^{bc}
	7.5	45.5^{a}	1.7^{a}	5.3^{a}	8.6^{c}	12.1^{a}	33.8^{c}	7.7^{a}	1.6^{c}
	10.0	45.0^{a}	3.1^{b}	6.4^{b}	7.0^{a}	11.7^{a}	32.3^{b}	9.6^{b}	1.2^{a}

Average values (n = 2). Values within a column with different superscript letters are significantly different (p <0.05); those with two superscripts are not significantly different from the figures with either of the same single superscripts.

Table 3. Extraction coefficients (%E_{PL}) of PC- and PI-enriched fractions obtained after fractionation of sunflower lecithin at different ethanol:water ratios and pHs of the partitioning solvent

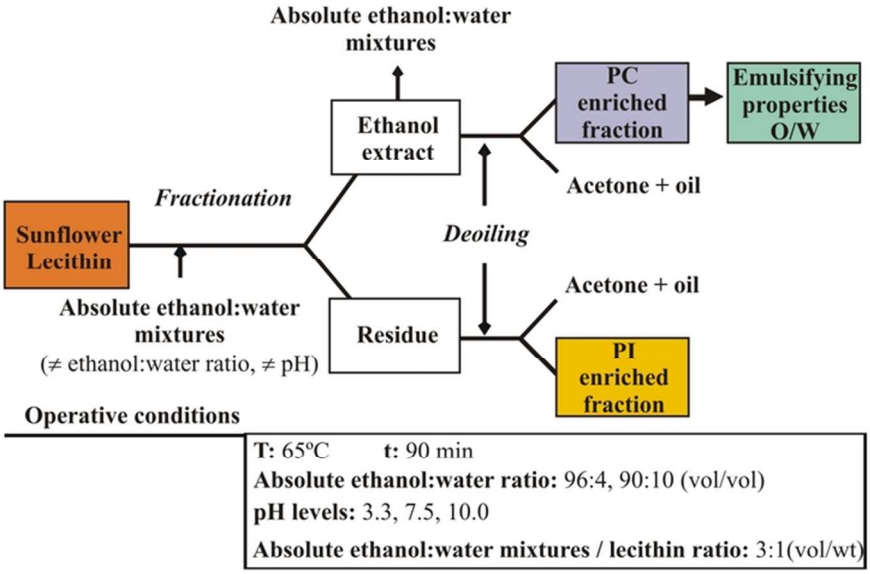
		PC-enriched fraction			PI-enriched fraction		
Ethanol:water [v/v]	**pH**	**%E_{PC}**	**%E_{PI}**	**%E_{PE}**	**%E_{PC}**	**%E_{PI}**	**%E_{PE}**
90:10	3.3	42.9^{a}	3.2^{c}	13.7^{a}	57.1^{c}	96.8^{a}	86.3^{c}
	7.5	44.4^{ab}	3.0^{bc}	13.7^{a}	55.6^{bc}	97.0^{a}	86.3^{c}
	10.0	48.0^{b}	3.5^{c}	14.9^{a}	52.0^{b}	96.5^{a}	85.1^{c}
96:4	3.3	55.2^{c}	2.4^{b}	19.1^{c}	44.8^{a}	97.6^{ab}	80.9^{a}
	7.5	54.2^{c}	1.6^{a}	17.8^{b}	45.8^{a}	98.4^{b}	82.2^{ab}
	10.0	54.8^{c}	3.0^{bc}	17.4^{b}	45.2^{a}	97.0^{a}	82.6^{b}

Average values (n = 2). Values within a column with different superscript letters are significantly different (p <0.05); those with two superscripts are not significantly different from the figures with either of the same single superscripts.

Table 4. De-Brouker mean diameter D [4,3] of the droplets in oil-water emulsions obtained with PC-enriched fractions as a function of the composition and pH of the extraction solvent

		D [4,3](µm)	
pH	**PC enriched fraction [% w/w]**	**Ethanol:water 90:10 [v/v]**	**Ethanol:water 96:4 [v/v]**
3.3	0.5	155.25^{e}	104.72^{c}
	1.0	90.22^{c}	53.15^{b}
	2.0	40.79^{a}	33.24^{a}
7.5	0.5	129.71^{d}	116.48^{c}
	1.0	60.99^{b}	66.07^{b}
	2.0	36.52^{a}	39.23^{a}
10.0	0.5	131.08^{d}	138.64^{d}
	1.0	64.69^{b}	63.86^{b}
	2.0	38.66^{a}	36.10^{a}

Average values (n = 2). Values within a column with different superscript letters are significantly different (p <0.05).



Graphical abstract
Flow diagram for the sunflower-lecithin extraction with ethanol:water mixtures at different pHs used for producing different PC-enriched fractions in order to analyze their emulsifying properties
70x48mm (300 x 300 DPI)

Graphical abstract legend:

Flow diagram for the sunflower-lecithin extraction with ethanol:water mixtures at different pHs used for producing different PC-enriched fractions in order to analyze their emulsifying properties