Chapter 25

Antioxidant and Emulsifying Properties of Modified Sunflower Lecithin by Fractionation with Ethanol-Water Mixtures

Dario M. Cabezas, Estefanía N. Guiotto,
Bernd W. K. Diehl and Mabel C. Tomás

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1. Introduction

Lecithins are a mixture of acetone insoluble phospholipids, containing mainly phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidic acid (PA), and other minor substances such as carbohydrates and triglycerides [1-3]. The production of sunflower oil in Argentina, is of utmost importance from an economic point of view [4]. In this country, sunflower lecithin could represent an alternative to soybean lecithin because it is considered a non-GMO product, which is in accordance with the preference of some consumers [5].

Lecithins, in native or modified state, are used in a wide range of industrial applications: dietetic, pharmaceutical, food, cosmetics, etc. This by-product of the oil industry represents a multifunctional additive for the manufacture of chocolate, bakery and instant products, margarine, mayonnaise, due to the characteristics of its phospholipids [6-8].

The introduction of changes in the relative concentration of the original phospholipid composition of lecithin can originate enriched fractions in certain phospholipids with different physicochemical and functional properties for diverse industrial purposes [9-11].

The fractionation process by ethanol or ethanol:water mixtures takes advantage of the different solubility of the phospholipids in this solvent. PC is readily soluble in ethanol whereas PI and PA are virtually insoluble. Phosphatidylethanolamine can be found in both fractions. This process can be carried out alone or in combination with other techniques such

as chromatography as a further purification step, especially for pharmaceutical, cosmetic and dietetic industry [12-13].

The main application of lecithin at the food industry is associated with its rol as emulsifying agent for dispersions or emulsions [14]. Emulsions are thermodynamically unstable systems from a physicochemical point of view. In virtue of that, it is important to characterize their behaviour against different destabilization processes (flocculation, coalescence, creaming, etc.) [15]. PC enriched fraction, due to its high PC/PE ratio and the lamellar phase structure of the PC at the interface between oil in water, is recognized to be a good oil-in-water (O/W) emulsifier [12,16,17].

On the other hand, PLs can contribute to an improvement of the oxidative stability of fats and oils. Various antioxidative mechanisms have been proposed for the phospholipid actions. For example, the amino functions of PC, PS, or PE, or the sugar moiety of PI have been shown to have metal-chelating properties and PC and PE presented a synergistic effect, with phenolic antioxidants such as tocopherols and flavonoids [18, 19].

The objective of this work was to evaluate the antioxidant and emulsifying properties of modified sunflower lecithins by fractionation with ethanol-water mixtures. In this sense, this study seeks to contribute to the food industry with useful information about developing tailor-made surface-active emulsifiers.

2. Materials and methods

2.1. Materials

Native sunflower lecithin was provided by a local oil industry (Vicentin S.A.I.C.).. This lecithin present a phospholipid composition of 43.1% (PC 16.2%, PI 16.5%, PE 5.3%, minor PLs 5.1%), 23.5% other compounds (glycolipids, complex carbohydrates), 33.4% oil.

Sunflower lecithin was deoiled with acetone, according to AOCS Official Method Ja 4-46, procedures 1–5 [20], obtaining the deoiled sunflower lecithin (DSL) (Figure 1). Then, DSL was stored at 0 ºC. This modified lecithin was used as control sample. Deoiling procedure was performed in duplicate.

2.2. Sunflower lecithin fractionation

Fractionation process was performed to 30 g of native sunflower lecithin with the addition of extraction solvents with different ethanol/water ratio (96:4, 100:0) using an ethanol/lecithin ratio of 3:1. These samples were incubated in a water bath at 65 °C during 60 min with moderate agitation (60 rpm), and then centrifuged at 1880 g, 10 ºC during 10 min. Afterwards, the corresponding ethanolic extracts were obtained and ethanol was eliminated by evaporation under vacuum [17].

Ethanol soluble phases were further deoiled with acetone, according to AOCS Official Method Ja 4-46, procedures 1–5, obtaining the different PC enriched fractions (PCF 96, PCF 100)

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(Figure 1). Then, both fractions were stored at 0 ºC. Fractionation and deoiling procedures were performed in duplicate.

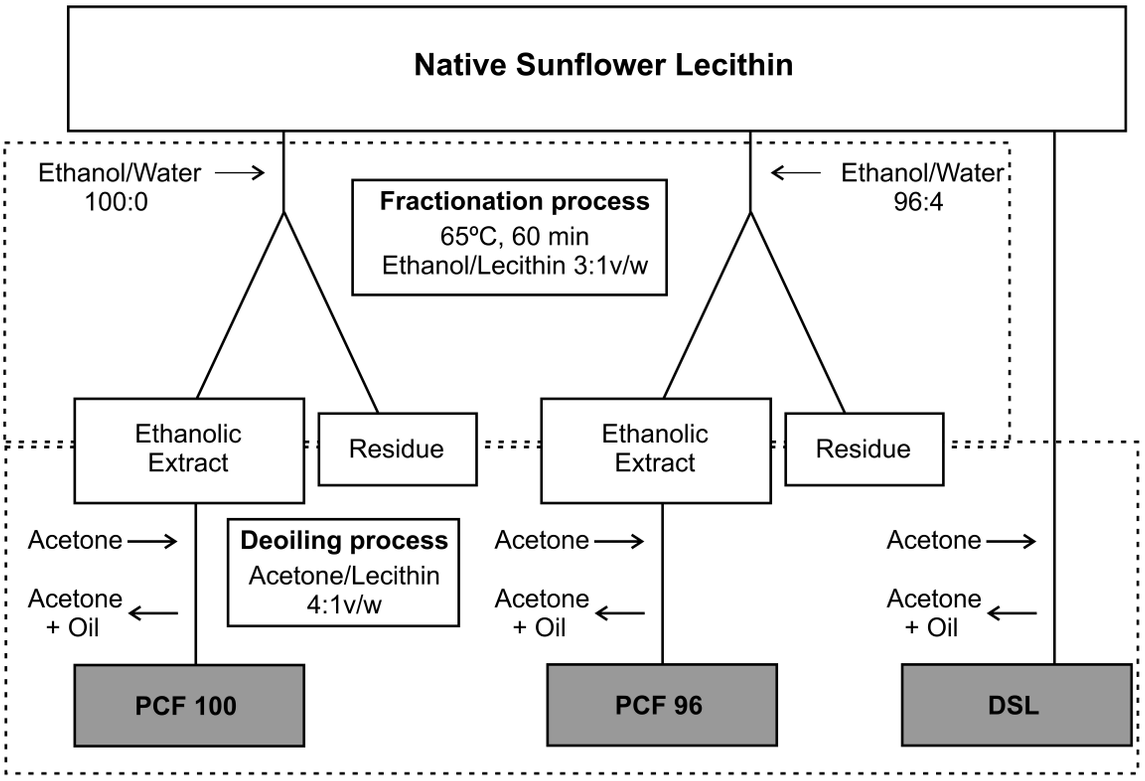

Figure 1. Flow diagram for deoiling and ethanol fractionation of native sunflower lecithin

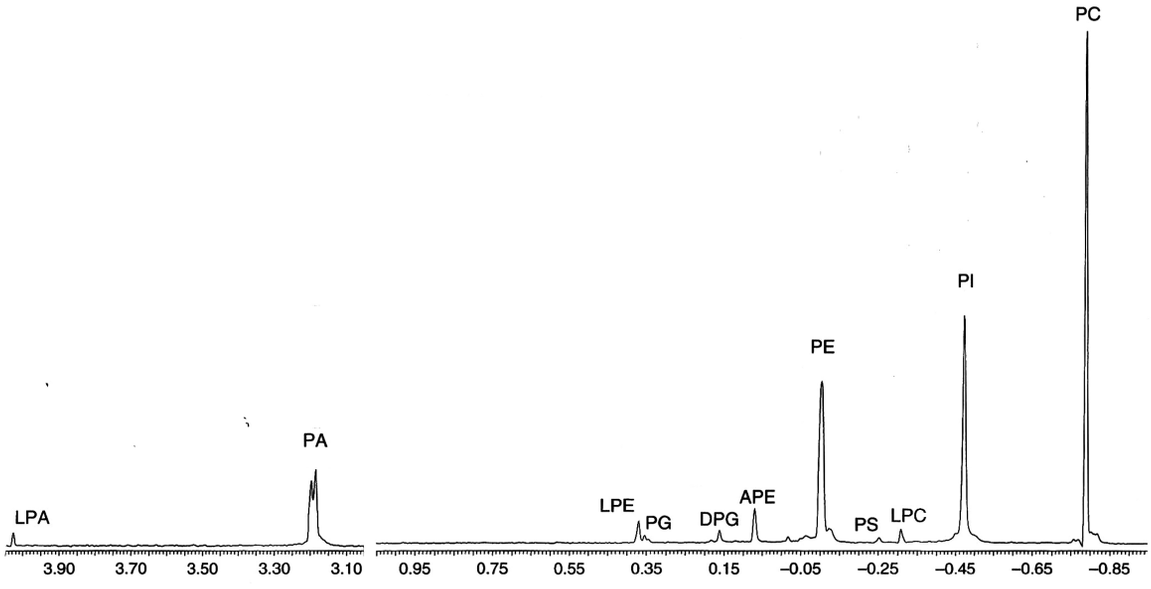

Figure 2. Spectrum of a phospholipidic sample

2.3. 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 internal standard (Spectral Service GmbH, Köln, Germany) (Figure 2) [21-23]. 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 of the different modified sunflower lecithins (MSLs) was expressed in terms of molar concentration (mol / 100 mol lecithin) [17].

2.4. Antioxidant properties

The antioxidant properties of the MSLs were evaluated by the Rancimat (Mod 679, Metrohm) method. 5 g of sunflower oil were added with different concentration of the analyzed samples (500-2000 ppm), heated at 98 °C, air flow 20 L/h. Stability was expressed as the induction time, according to Gutiérrez [24].

Also, the highest antioxidant concentration was selected for each modified lecithin. The same procedure was carried out with previous thermal treatments at 120 and 160 °C for 1 h, adding the modified lecithins before heating. Refined sunflower oil with and without previous heat treatments were used as control samples.

Oil tocopherol content was determined by normal phase HPLC using a Hewlett Packard chromatography system (HPLC Hewlett Packard 1050 Series, Waldbronn, Germany) equipped with a fluorescence detector Agilent 1100 Series (Agilent Technology, Palo Alto, CA, USA) following the procedures described in IUPAC 2.432 [25] and AOCS Ce8-89 [20].

2.5. Oil-in-Water (O/W) emulsions preparation

Refined 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 Cabezas et al. [26] with the addition of MSLs in a range of 0.1-2.0% (wt/wt).

2.6. Optical characterization of emulsions

The backscattering of light was measured using a QuickScan Vertical Scan Analyzer (Coulter Corp., Miami, FL). The backscattering of monochromatic light (λ = 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 60 min. This methodology allowed to discriminate between particle migration (sedimentation, creaming) and particle size variation (flocculation, coalescence) processes [27]. The basis of the multiple light scattering theory has been exhaustively studied by Mengual et al. [28].

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2.7. Statistical analysis

Data were evaluated by analysis of variance (ANOVA) using the software Systat® 12.0 [29]. For this purpose, differences were considered significant at $p < 0.05$.

3. Results

3.1. Phospholipid composition

The quantitative analysis of phospholipids was performed by ^{31}PNMR which represents a modern and the most sophisticated methodology for evaluating the composition of lecithins, since it is possible to obtain a separate signal for each phospholipid class [9, 21]. The results presented in Table 1, evidenced the high solubility of the phosphatidylcholine in the different ethanolic solvents assayed. ^{31}PNMR determinations of the different enriched PC fractions (PCF 96 and PCF 100) exhibited an important concentration of PC (>71.0 %) as well as low values of PI (<8%) in comparison with the native and deoiled sunflower lecithin assayed.

3.2. Rancimat analysis

The oxidative stability of the refined sunflower oil (control) and the activity of different modified lecithins were evaluated determining the induction time (t_i) by a Rancimat analysis. This methodology can be used to evaluate the efficiency of various synthetic or natural antioxidants to stabilize fats and oils against an accelerated oxidation test [30]. Figure 3 shows t_i for the modified sunflower lecithins used at different concentrations.

PL	DSL [a]	PCF 96 [a]	PCF 100 [a]
PC	36.7	71.2	76.3
1-LPC	< 0.1	< 0.1	< 0.1
2-LPC	1.5	3.3	2.8
PI	35.3	7.4	3.6
LPI	< 0.1	< 0.1	< 0.1
PE	15.1	12.2	10.6
LPE	< 0.1	0.8	0.4
PA	5.0	1.3	1.4
LPA	< 0.1	< 0.1	< 0.1
Others PL	6.4	3.8	4.9

[a] Values represent means (n = 2). The coefficient of variation was lower than 6%

Table 1. Percentage phospholipid composition (mol PL / mol total PL) of deoiled sunflower lecithin and PC-enriched fractions analyzed by ^{31}PNMR

The antioxidant addition increased the t_i of the control as a function of increasing concentration. MSLs did not show a marked difference of the respective t_i values at concentration of 500 ppm. However, at high concentrations (1000 - 2000 ppm), both PC fractions had a high significant effect (p <0.01) on the oxidative stability of the control system, in relation to the values recorded by DSL addition. In this sense, 2000 ppm of DSL increased the t_i value of the control oil of 49.1%, while similar concentrations of PCF 96 and PCF 100 enhanced this value 167.4% and 108.3%, respectively (Table 2).

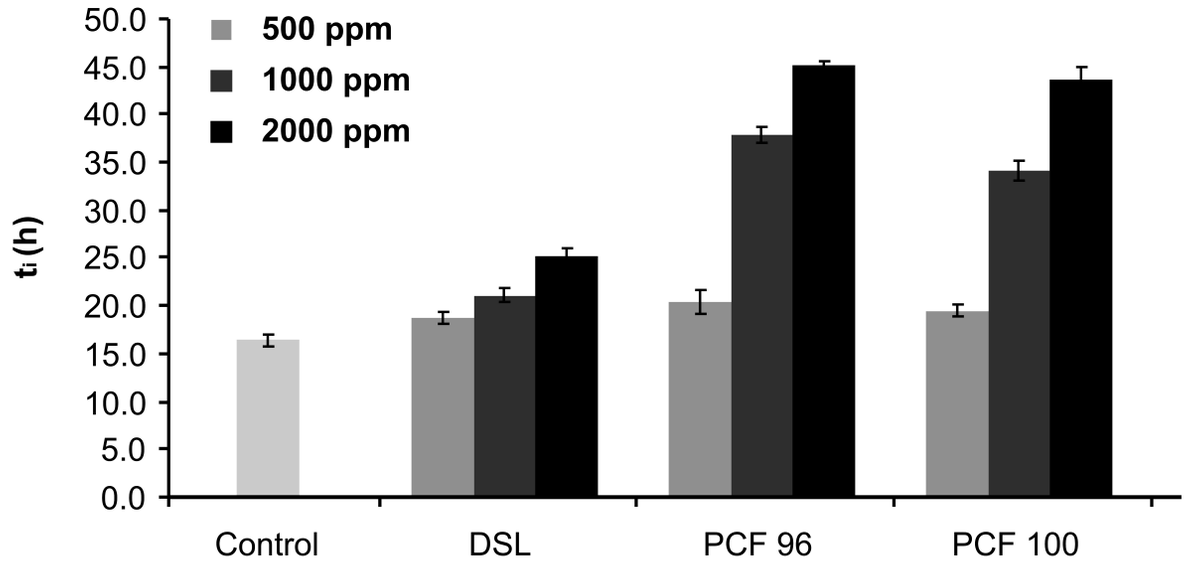

Figure 3. Induction times (ti) of refined sunflower oil (control) added with different concentrations of modified sunflower lecithins (Rancimat Mod 679, Metrohm). Mean values (n = 3) ± sd

	Δ ti WT / ti control WT (%)	Δ ti T120 / ti control T120 (%)	Δ ti T160 / ti control T160 (%)	Δ ti T120 / ti control WT (%)	Δ ti T160 / ti control WT (%)
Control	0.0	0.0	0.0	-26.6	-25.8
DSL	49.1	56.9	19.0	15.1	-11.7
PCF 96	167.4	211.9	113.9	128.8	58.6
PCF 100	157.7	177.5	95.4	103.6	44.9

control, refined sunflower oil; WT, samples without thermal pre-treatment; T120, samples with thermal pre-treatment 120°C, 1h; T160, samples with thermal pre-treatment 160°C, 1h

Table 2. Percentage induction times increase of refined sunflower oil added with different modified sunflower lecithins (MSLs) with or without thermal pre-treatment

Induction times of oils added with the different MSLs without thermal pre-treatments, showed higher values than those obtained by Pan et al. [31], who reported under similar test conditions that 2000 ppm of native sunflower lecithin produced a ti increase of 12%. The in-

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troduction of changes in the original phospholipids concentration of this native lecithin by different modification processes (deoiling, ethanol fractionation) allow to obtain modified lecithins with better physicochemical and functional properties with respect to the starting material.

A concentration of 2000 ppm of the different modified lecithins showed the highest antioxidant activity in this assay. Therefore, refined sunflower oil added with this concentration of MSLs was previously subjected to different heat treatments at 120 and 160 ° C for 1 h. The ti values of the oils added with PCF samples had a high significant difference (p <0.01) in relation to those added with DSL (Table 2). PC enriched fractions reduced the negative effect of the heat treatments over the oxidative stability of refined sunflower oil. These pre-treated samples showed higher ti values respect to the control system, even without thermal pre-treatment. In this sense, oils added with PCF 96 and PCF 100, subjected to a heat treatment at 160°C presented induction times of 58.6 and 44.9% higher than the untreated oil, respectively.

It is interesting to note that PCF 96 recorded the best antioxidant capacity producing an increase of 211.9% (120°C, 1h) and 113.9% (160°C, 1h) in relation to those recorded for the respective control oils. This fact indicates that the fractionation process carried out with ethanol 96 ° not only allows to obtain fractions enriched in PC with antioxidant activity, but also this activity is greater than those exhibited using DSL and the PCF obtained through fractionation with absolute ethanol (PCF 100).

The ability of phospholipids to inhibit lipid oxidation in bulk oils has been known for several decades, but the mechanism of stabilization still remains controversial [32]. However, many research works have proposed different antioxidant mechanisms for these compounds. Particularly, PC and PE have been shown to have metal-chelating and scavenging properties. Also, this type of phospholipids present a synergistic effect with the different tocopherols (α-tocopherol 512.84 μg/g; β-tocopherol 4.55 μg/g) regenerating the oxidized tocopherol molecule by donation of a hydrogen atom of their amino function [18, 33]. This fact and the high PC and PE concentrations are in correlation with the better antioxidants characteristics observed for both PCF assayed.

3.3. 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). These profiles were analysed in different zones of the emulsion: Zone I (10-20 mm) to visualize the destabilization process by migration of the oil droplets from the bottom towards the top of the tube (creaming), Zone II (40-45 mm) characterized by the accumulation of oil droplets after the creaming process (cream phase) and Zone III (50-60 mm), to analyze the destabilization of the cream phase [34]. For instance, Figure 4 shows a typical profile obtained for an O/W emulsion with the addition of 0.5% of DSL.

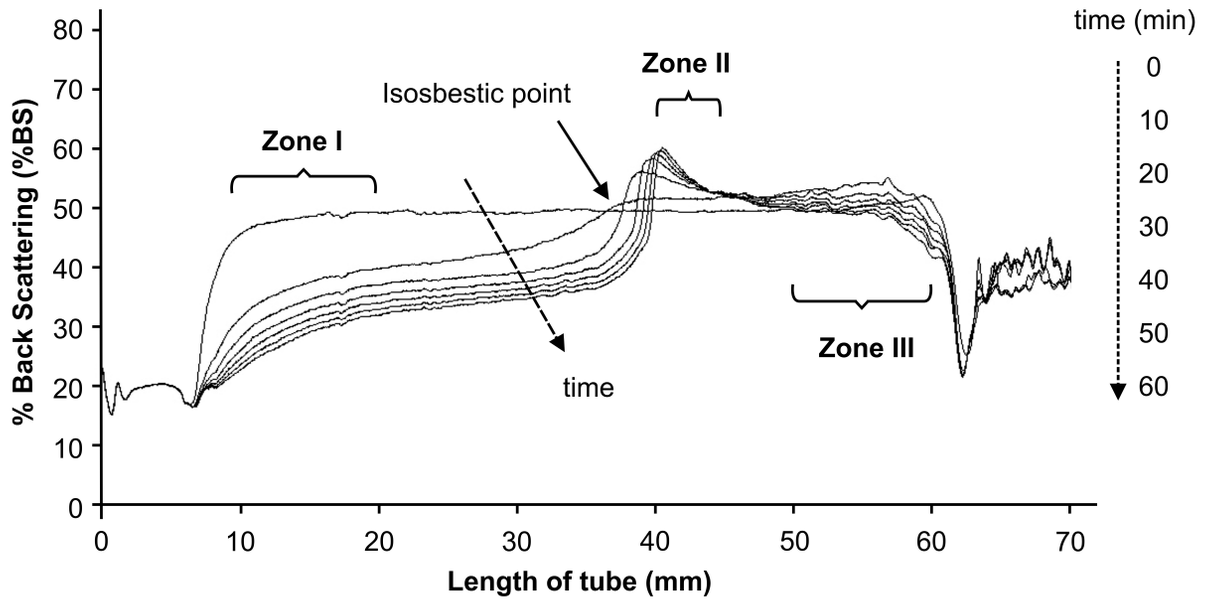

Figure 4. Backscattering (%BS) profile of a O/W emulsion (30:70 wt/wt) with the addition of 0.5% of DSL

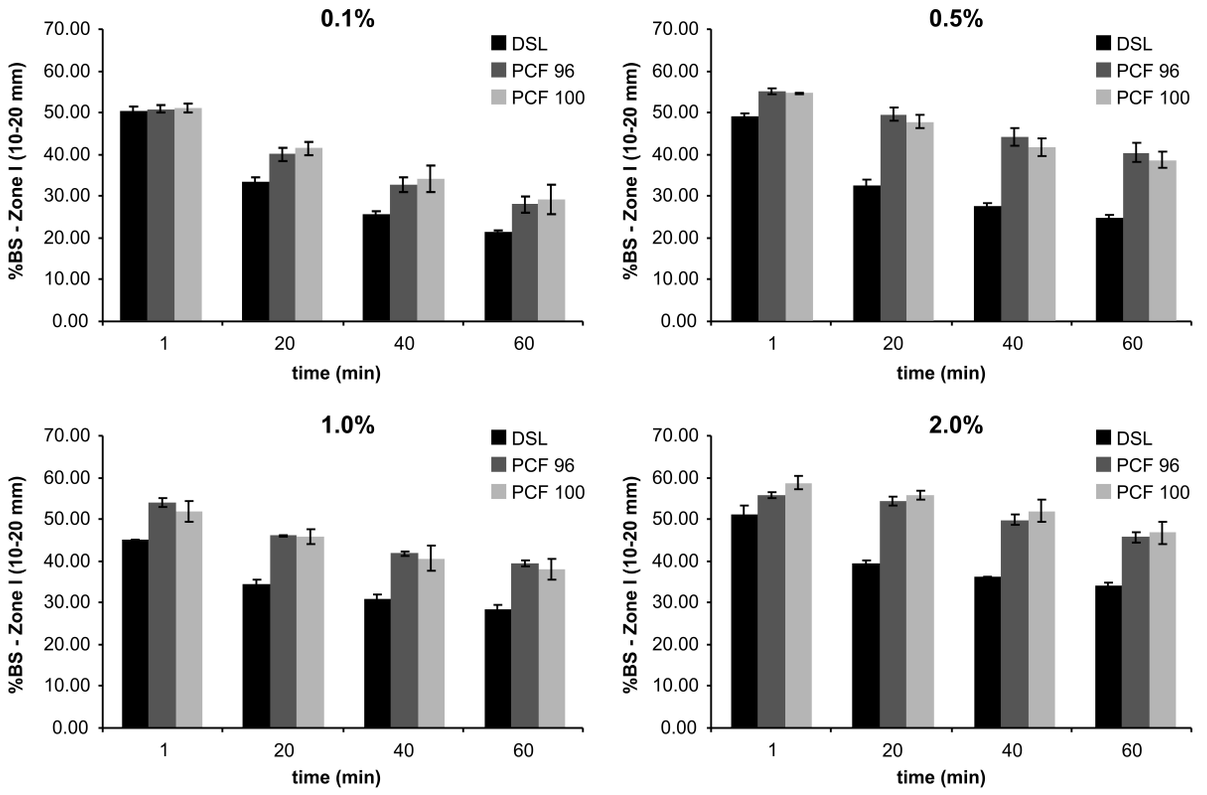

Figure 5. Backscattering (%BS) values of O/W emulsions (30:70 wt/wt) with the addition of different modified sunflower lecithins, Zone I (10-20 mm). Mean values (n = 3) ± sd

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The creaming destabilization process (i.e. migration of oil particles to the upper portion of the tube) is evidenced by a decrease of %BS values at the bottom of the tube and the appearance of an isosbestic point [34]. This point separates the zones with lower (left) and higher (right) values than the initial %BS (Figure 3). 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 (Figure 5). In particular, the PC enriched fractions (PCF 96 and PCF 100) produced a high stability in O/W emulsions than DSL, over the range of concentration studied. In this sense, it should be noted that QuickScan profiles of the different PCF did not show significant variations of %BS values for 2.0% during 40 minutes. These results are in concordance with those previously reported by Wu and Wang [12], related to the emulsifying activity of PC enriched fractions from soy lecithin.

Moreover, O/W emulsions with 0.1-0.5% of DSL showed a sharp decrease of %BS in the Zone I. This behaviour indicates a rapid destabilization of these emulsions by creaming.

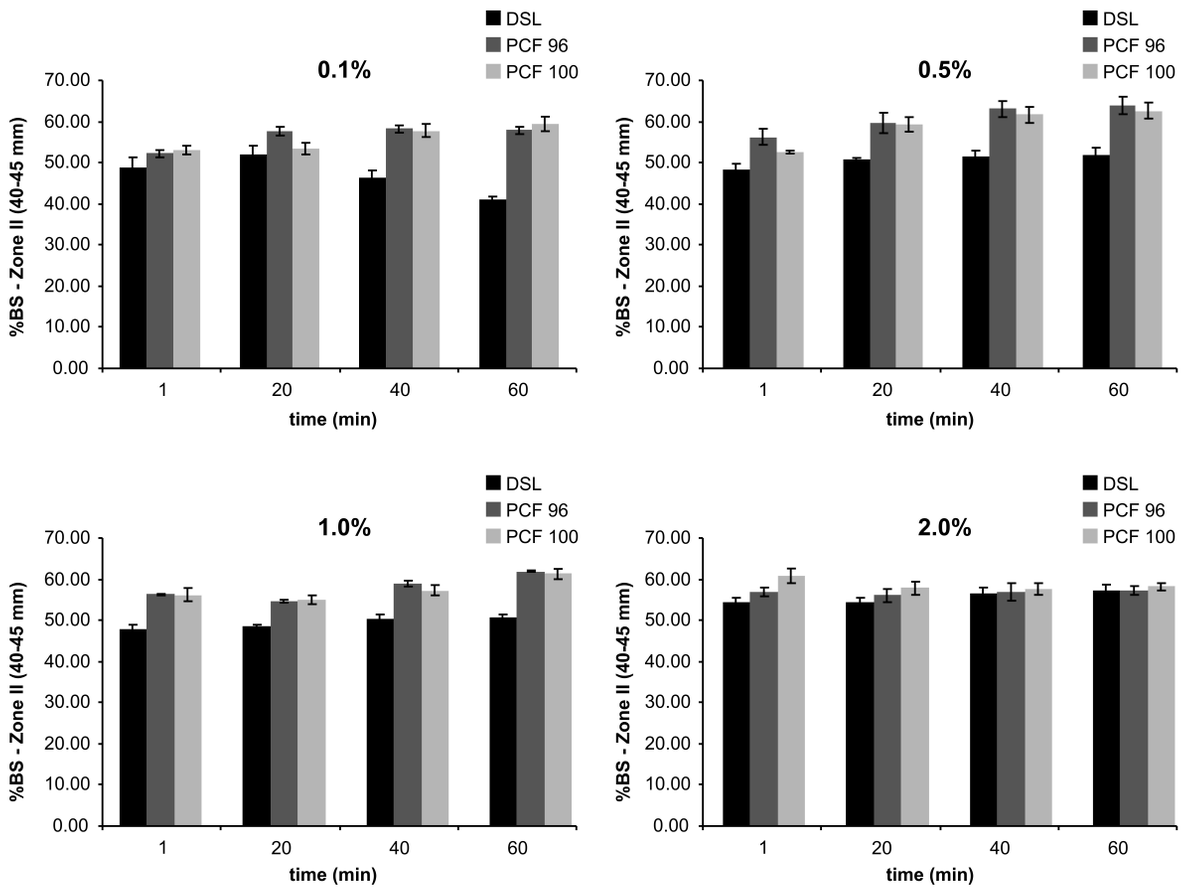

Figure 6. Backscattering (%BS) values of O/W emulsions (30:70 wt/wt) with the addition of different modified sunflower lecithins, Zone II (40-45 mm). Mean values (n = 3) ± sd

The tube zone between 40-45 mm (Zone II) is characterized by the accumulation of oil droplets after the creaming process (cream phase). Figure 6 shows the %BS values vs. time in Zone II. Emulsions formulated with PC enriched fractions presented higher %BS values than those obtained using DSL, for all concentrations studied. The higher levels of %BS and the greater stability of these emulsions formulated with a high concentration of phosphatidylcholine would be associated with the formation of dense cream phases with a lower proportion of continuous phase inside [26].

The stability of the different cream phases were analyzed by the evolution of the % BS values vs. time in Zone III (50-60 mm) (Figure 7). %BS values remained constant at concentrations of emulsifier above 0.1% of the different modified lecithins. This behaviour confirms the stability provided by these MSLs against the coalescence process, in these conditions.

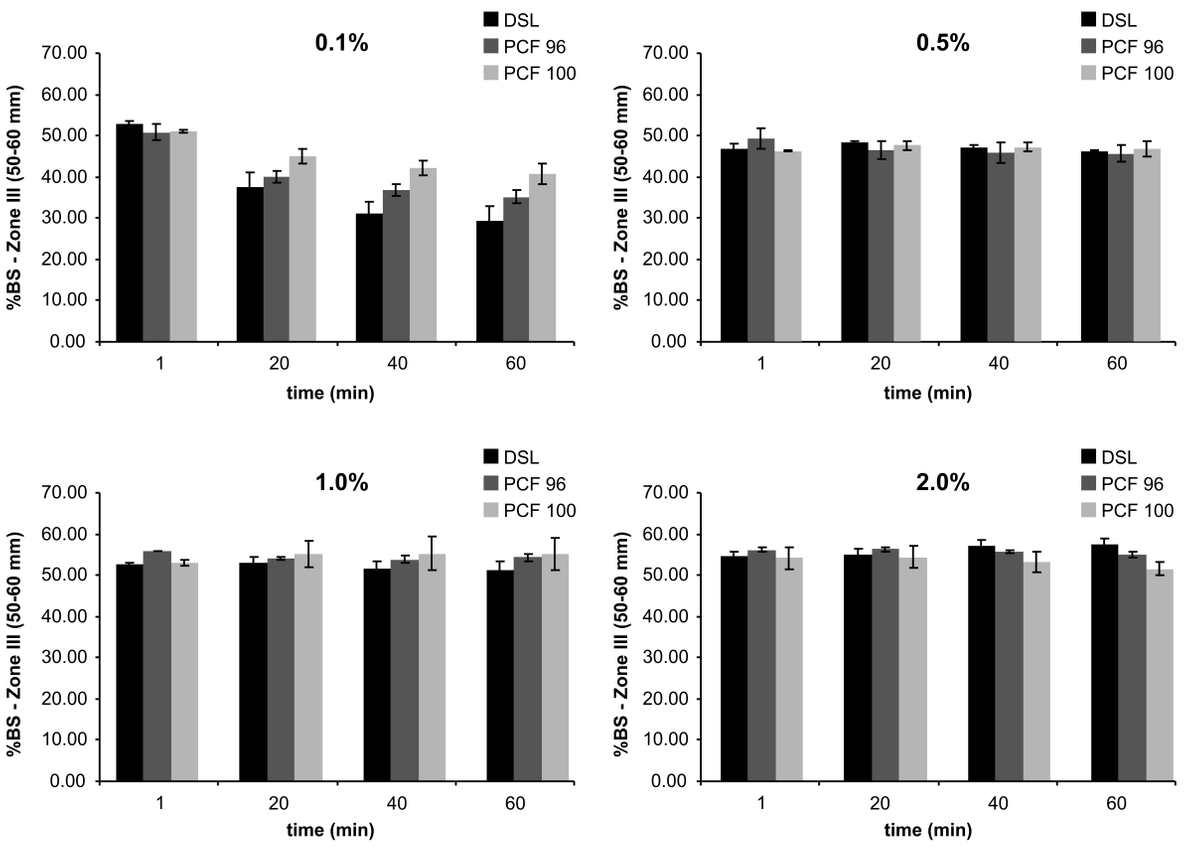

Figure 7. Backscattering (%BS) values of O/W emulsions (30:70 wt/wt) with the addition of different modified sunflower lecithins, Zone III (50-60 mm). Mean values (n = 3) ± sd

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However, emulsion with 0.1 of DSL did not allow the formation of the cream phase. These results are related to the rapid decrease of %BS, the formation of an oil layer in the upper part of the tube and the absence of an isosbestic point (data not shown) ; suggesting the occurrence of a cream phase destabilization by coalescence [27].

The hydrophilic-lipophilic balance value (HLB) is often used in order to explain the performance of emulsifiers [15]. The high concentration of phosphatidylcholine (hydrophilic phospholipid) increases this empirical value in the PC enriched fractions. In this sense, the best properties of these modified lecithins as emulsifying agents in O/W emulsions was according with Carlsson [35], who has determined that lecithin with high HLB values present best O/W emulsifying properties.

Also, the phase structure at the interface of the different phospholipids influences the emulsion formation and stability [16]. PC forms a lamellar phase at the interface between oil and water with well ordered mono- and bi- layers. This structure has a great importance for the stabilisation of O/W emulsions.

4. Conclusions

The study of the induction times showed a highly significant difference (p <0.01) between the antioxidant activity exhibited by the different PCF in relation to the addition of DSL. Also, these fractions allowed to obtain more stable O/W emulsions (30:70 wt/wt) in comparison with those added with DSL at different concentrations (0.1-2.0%) in terms of kinetic destabilization as a function of changes in the backscattering values vs. time. These results showed that PC enriched fractions (PCF 96 and PCF 100) constitute a potential alternative as emulsifier agent for the food industry.

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Author details

Dario M. Cabezas[1], Estefanía N. Guiotto[1], Bernd W. K. Diehl[2] and Mabel C. Tomás[1*]

*Address all correspondence to: mabtom@hotmail.com

1 Centro de Investigación y Desarrollo en Criotecnología de Alimentos (CIDCA) – CCT La Plata – CONICET - Facultad de Ciencias Exactas, Universidad Nacional de La Plata (FCE - UNLP), La Plata, Argentina

2 Spectral Service GmbH Laboratorium für Auftragsanalytik, Cologne, Germany

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