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Sunflower Lecithin: Application of a Fractionation Process with Absolute Ethanol

D. M. Cabezas · B. W. K. Diehl · M. C. Tomás

Abstract Native or modified lecithins are widely used as a multifunctional ingredient in the food industry. A fractionation process of sunflower lecithin (a non GMO product) with absolute ethanol was used for obtaining enriched fractions in certain phospholipids under different experimental conditions (temperature 35–65 °C, time of fractionation 30–90 min, ethanol/lecithin ratio 2:1, 3:1). Phospholipid enrichment in PC and PI fractions was obtained and analyzed by ^{31}P NMR determinations. The percent extraction coefficients for different phospholipids ($\%E_{PC}$, $\%E_{PE}$ and $\%E_{PI}$) in both fractions were calculated. Values of $\%E_{PC}$ in PC fractions significantly increased ($p < 0.05$) from 12.8 (35 °C, 30 min, 2:1) to 57.7 (65 °C, 90 min, 3:1) at increasing temperature and incubation time. $\%E_{PE}$ varied from 3.0 to 18.3 in the same fraction while $\%E_{PI}$ presented lower values (<3%) under all the conditions assayed. The study of the effect of the operating conditions on the fractionation process evidenced a relevant influence of temperature, incubation time and to a minor extent of the ethanol/lecithin ratio on the enriched fraction yield% and selectivity of the main phospholipids (PC, PI, PE) estimated by $\%E_{PL}$. Response surface methodology (RSM) was utilized to explain the influence of the different parameters to optimize this process.

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Introduction

Lecithin is a mixture of acetone insoluble phospholipids, containing mainly phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), minor compounds such as phosphatidic acid (PA) and other substances (triglycerides, carbohydrates, etc.). It has been widely used in the nutritional, pharmaceutical and cosmetic industries [1–3] Food technology mainly includes natural or modified lecithins in many processes due to their versatile role as emulsifiers, viscosity regulators, anti-spattering and dispersing agents. Their applications are associated with the manufacture of bakery products, chocolate, milk powder, margarines, mayonnaise [4–7].
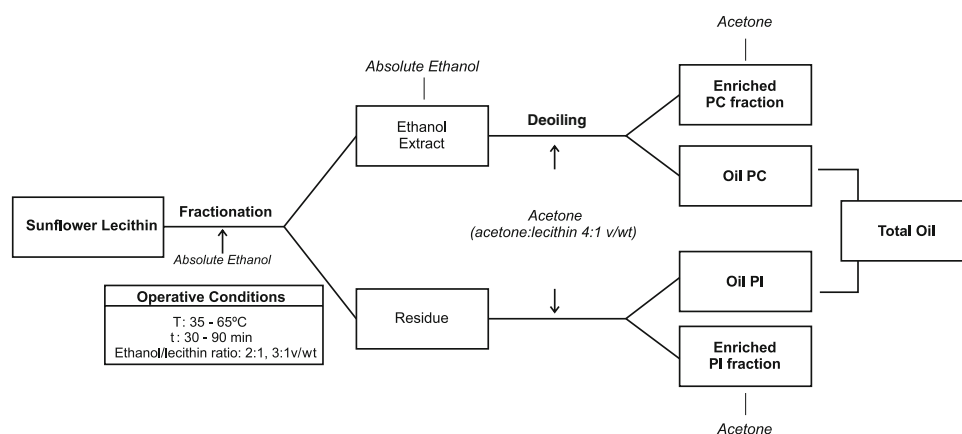
Surface activity and performance of commercial lecithin can be improved by modification processes such as fractionation with alcohols based on the different solubility of phospholipids in this medium as other authors and patents have described [5, 8–12]. Thus, it is possible to make changes in the relative concentration of phospholipids of the original lecithin leading to enriched fractions in certain species of phospholipids. Then, new products with different physicochemical and functional characteristics are produced [13].

The lecithin fraction that is soluble in ethanol is enriched in phosphatidylcholine and is very useful as an emulsifier of oil in water emulsions. On the other hand, the ethanol insoluble fraction, that is enriched in phosphatidylinositol and phosphatidylethanolamine, is characterized as a good water in oil emulsifying agent [14, 15].

Taking into account the processing conditions, enriched fractions with different PC/PE ratios are likely to be produced for different purposes such as margarine manufacture, special emulsifiers and pharmaceutical formulations.

In Argentina, the production of sunflower oils is of utmost importance, this fact being of great economic relevance [16]. Sunflower lecithin—a byproduct of the degumming processing of oils—[17–19] is an alternative to soybean lecithin because it is considered a non GMO product which is currently preferred by certain consumers. Although, some authors have made contributions to the study of sunflower lecithin, most of them concern phospholipid composition, physicochemical properties or some functional characteristics [14, 20, 21]. Thus, it seems that modification processes such as fractionation have not been extensively applied to this type of lecithin.

The aim of this work was to evaluate the influence of the main operating conditions on a laboratory scale on the application of a fractionation process to sunflower lecithin in order to obtain different enriched fractions.

Material and Methods

Materials

Sunflower lecithin was provided by a local oil company.

Sunflower Lecithin Fractionation

The sunflower lecithin used as starting material presents a phospholipid composition of 43.1% (PC 16.2%, PI 16.5%, PE 5.3%, minor phospholipids 5.1%), 23.5% other compounds (glycolipids, complex carbohydrates), 33.4% oil. The fractionation process was carried out on sunflower lecithin with the addition of absolute ethanol. Different

operative parameters such as temperature, time of incubation and ethanol/lecithin ratio (2:1, 3:1) were analyzed according to yield and phospholipid composition of the different fractions obtained. Samples of 30 g were incubated in a water bath in a range of 35–65 °C, 30–90 min with moderate agitation and then centrifuged at 1880 g, for 10 min, at 10 °C. Afterwards, the corresponding ethanolic extracts and residues were obtained for each condition and the ethanol was eliminated by evaporation under vacuum.

Ethanol soluble and insoluble phases (residues) were further deoiled with acetone, according to AOCS Official Method Ja 4–46, procedures 1–5 [22] obtaining the enriched PC and PI fractions, respectively. Then, both fractions were stored at 0 °C. (Fig. 1). Fractionation procedures were performed in duplicate.

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

Enriched fraction Yield (%)

$$= \frac{\text{amount of fractionated sunflower lecithin}}{\text{amount of starting sunflower lecithin}} \times 100 \quad (1)$$

Also, the following equation must be considered:

PC enriched fraction Yield (%)
+ PI enriched fraction Yield (%) + % Oil
= 100% $\quad (2)$

Phospholipid Composition

Sample Preparation

A 100 mg amount from each sample was diluted in 1 mL deuterated chloroform, 1 mL methanol and 1 mL Cs-EDTA. The organic layer was separated after 15 min shaking and analyzed by ^{31}P NMR.

Quantitative ^{31}P NMR analyses were carried out with a Bruker Avance 300 MHz automatic spectrometer using triphenyl phosphate as an internal standard (Spectral Service GmbH, Köln, Germany) [23, 24].

Phospholipid content (g PC/100 g of each fraction (%PC), g PI/100 g of each fraction (%PI) and g PE/100 g of each fraction (%PE)) of samples obtained under the different conditions of the fractionation process were determined by this spectroscopic technique.

Data Evaluation

The differential fractionation of each phospholipid in absolute ethanol was followed by calculating the corresponding extraction coefficient $\%E_{PL}$ ($\%E_{PC}$, $\%E_{PE}$ and $\%E_{PI}$) for both types of enriched fractions.

$\%E_{PL}$(PC enriched fraction)

$$= \frac{m_{PL}(\text{PC enriched fraction})}{m_{PL}(\text{PC enriched fraction}) + m_{PL}(\text{PI enriched fraction})}$$
$$\times 100 \tag{3}$$

where PL: PC, PE or PI

m_{PL} (PC enriched fraction) = PC enriched fraction yield% $\times$ % PL (PC enriched fraction)

m_{PL} (PI enriched fraction) = PI enriched fraction yield% $\times$ % PL (PI enriched fraction)

The expression in Eq. 4 must be considered for calculations:

$\%E_{PL}$(PC enriched fraction)

$$+ \%E_{PL}(\text{PI enriched fraction}) = 100\% \tag{4}$$

Statistical Analysis

Data were evaluated by the analysis of variance (ANOVA). Differences were significant at $p < 0.05$.

Response surface methodology (RSM) was performed to optimize the fractionation process in order to obtain the maximum extraction coefficient $\%E_{PC}$ in enriched PC fraction [25]. Response surface curves and contour plots were obtained according to an additive model for the different processing conditions using MATLAB$^{\circledR}$. For this purpose, variables were normalized within the range of -1 to 1.

Results and Discussion

The PC and PI enriched fractions yield% obtained under the different processing conditions assayed during the fractionation of sunflower lecithin is shown in Fig. 2. A significant increment ($p < 0.05$) as a function of the increase of temperature for PC enriched fractions yield% was noted. Contrary behavior was recorded for the PI enriched fraction yield%. Similar effects, though less intense, were observed as a function of the incubation time and ethanol/lecithin ratio. It was found that temperature strongly affected this process when compared to the other
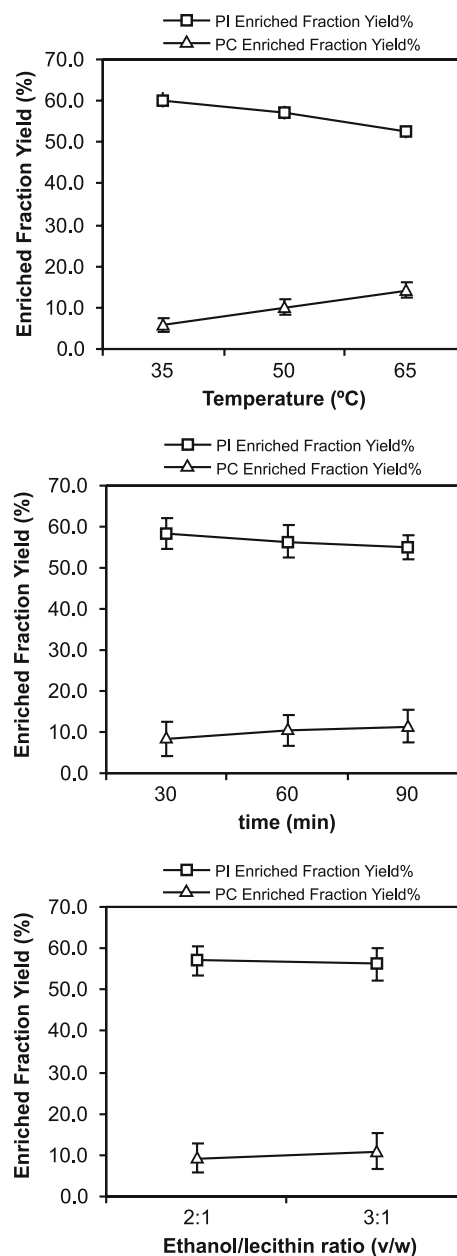

Fig. 2 Enriched fractions yield% obtained by fractionation of sunflower lecithin under different operating conditions: –□– PI enriched fraction yield, –△– PC enriched fraction yield. Error bars represent SD

variables. This fact was also related to the low level of standard deviation observed (Fig. 2). This information is in agreement with the experimental results reported in previous works dealing with the fractionation of soybean lecithins where a marked relationship between temperature—ethanol extract yield was recorded [13].

Furthermore, the effect of ethanol fractionation on the oil of starting lecithin was analyzed in order to elucidate further industrial applications. Thus, the oil distribution was evaluated as displayed in Fig. 1. The sunflower

Table 1 Influence of the operating conditions on the oil distribution during the fractionation process of sunflower lecithin for the PC enriched fraction

Run	Ethanol/lecithin ratio (v/w)	t (min)	T (°C)	% Oil PC/ethanol extract[a,b]	% Oil PC/total oil[a,b]
1	2:1	30	35	76.0	31.6
2	2:1	30	50	67.5	47.6
3	2:1	30	65	63.0	61.4
4	2:1	60	35	69.3	38.9
5	2:1	60	50	60.3	48.4
6	2:1	60	65	60.9	58.8
7	2:1	90	35	70.7	42.5
8	2:1	90	50	59.9	48.8
9	2:1	90	65	58.3	55.9
10	3:1	30	35	73.1	37.9
11	3:1	30	50	62.8	43.7
12	3:1	30	65	60.6	65.9
13	3:1	60	35	68.8	45.3
14	3:1	60	50	65.6	55.5
15	3:1	60	65	59.3	70.9
16	3:1	90	35	65.6	48.0
17	3:1	90	50	60.8	62.1
18	3:1	90	65	58.9	73.6

[a] see Figure 1

[b] Values represent means (n = 2). The coefficient of variation was lower than 5%

Table 2 Phospholipid composition of different fractions obtained by fractionation of sunflower lecithin under different operating conditions by ^{31}P NMR[a]

Run	Independent variables			PC enriched fraction[b]			PI enriched fraction[b]		
	Ethanol/lecithin ratio	t (min)	T (°C)	%PC	%PI	%PE	%PC	%PI	%PE
1	2:1	30	35	54.3	2.5	5.6	20.1	28.4	9.8
2	2:1	30	50	51.9	2.6	6.0	17.7	28.6	9.8
3	2:1	30	65	50.3	2.9	7.0	14.0	32.0	9.8
4	2:1	60	35	54.6	2.3	5.6	17.9	28.3	9.7
5	2:1	60	50	53.2	2.2	6.1	15.9	29.6	9.5
6	2:1	60	65	51.3	2.6	6.8	13.3	32.0	9.7
7	2:1	90	35	54.0	2.3	5.7	18.4	29.6	10.1
8	2:1	90	50	53.6	2.1	6.1	15.3	30.7	10.2
9	2:1	90	65	47.5	2.2	6.1	12.5	30.3	9.9
10	3:1	30	35	51.2	2.3	5.1	19.2	28.5	9.7
11	3:1	30	50	50.3	3.0	6.1	18.0	29.1	9.8
12	3:1	30	65	45.6	2.9	6.0	14.7	32.1	9.6
13	3:1	60	35	51.6	2.0	4.9	18.1	28.8	9.4
14	3:1	60	50	51.4	2.7	6.2	15.6	30.2	9.9
15	3:1	60	65	47.9	2.5	6.6	12.9	33.3	10.0
16	3:1	90	35	52.5	2.0	4.9	17.3	30.0	10.1
17	3:1	90	50	52.2	2.4	5.8	14.6	31.4	9.8
18	3:1	90	65	49.4	2.5	6.4	12.2	33.9	9.7

[a] Values represent means (n = 2)

[b] %PL: g PL/100 g of each fraction

lecithin used as starting material presented an oil content of 33.4%. The evolution of the % Oil PC/ethanol extract and the Oil PC/total oil ratios is shown in Table 1. An increase in the levels of operative parameters produced a diminution of the Oil PC with respect to the ethanol extract due to an increase in the efficiency of PC extraction. Also, Oil PC
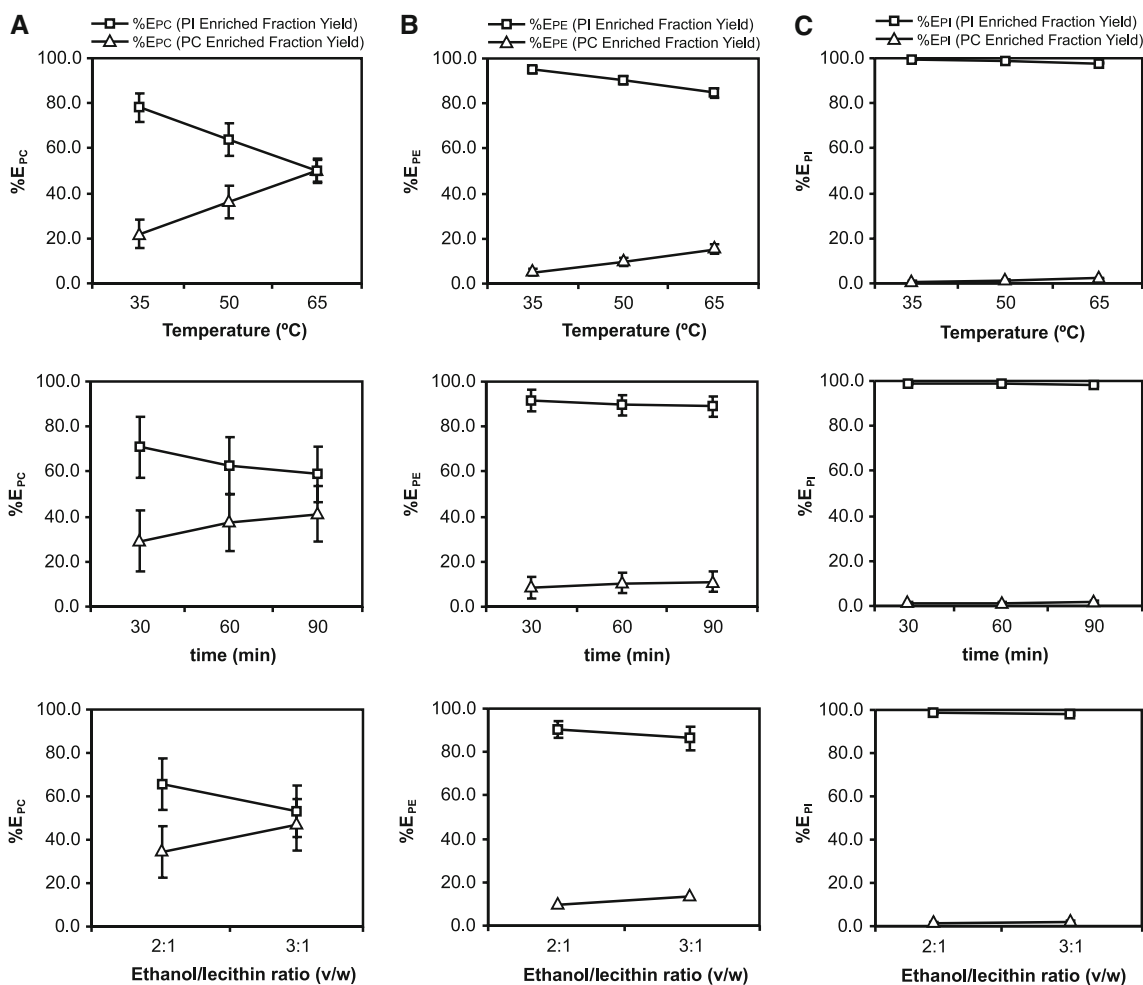
Fig. 3 Percent extraction coefficient of PC enriched fractions obtained by fractionation of sunflower lecithin under different processing conditions (temperature, time, ethanol/lecithin ratio): **a**: –□– %E_{PC} (PI enriched fraction yield), –△– %E_{PC} (PC enriched fraction yield), **b**: –□– %E_{PE} (PI enriched fraction yield), –△– %E_{PE} (PC enriched fraction yield), **c**: –□– %E_{PI} (PI enriched fraction yield), –△– %E_{PI} (PC enriched fraction yield). Error bars represent SD
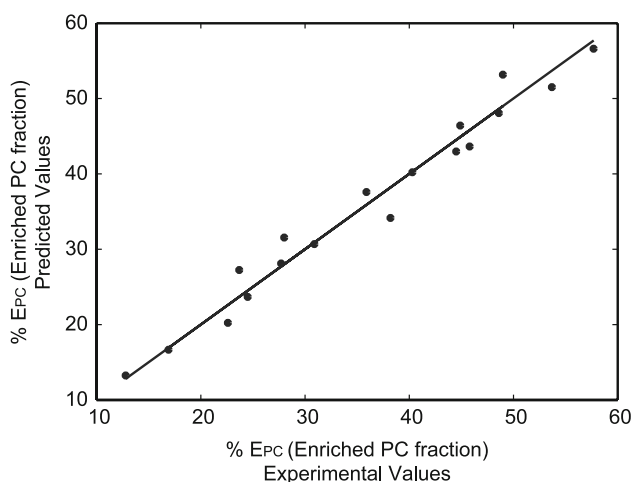

Fig. 4 Correlation between predicted and experimental values of %E_{PC} in the PC enriched fraction obtained by the fractionation process of sunflower lecithin

and Oil PI content presented significant changes ($p < 0.05$) as a function of temperature, ethanol/lecithin ratio and time of process (mainly ranging from 30 to 60 min). The results showed that Oil PC represented 73.6% from the total oil content at 65 °C, 90 min, ethanol/lecithin 3:1 and 31.6%, at 35 °C, 30 min, ethanol/lecithin ratio 2:1, respectively. As shown, the relationships in Table 1 presented an opposite behavior. This fact could be attributed to the increase of the enriched PC fraction yield% at the highest levels of operating conditions. This rise was found to be more important than that in Oil PC which resulted from its high solubility under the present experimental conditions. Then, the best PC enriched fraction yield% was obtained under similar conditions to achieve the major oil extraction.

The characterization of the different enriched PC and PI fractions in terms of phospholipid composition was performed by ^{31}P NMR. This technique is currently one of the
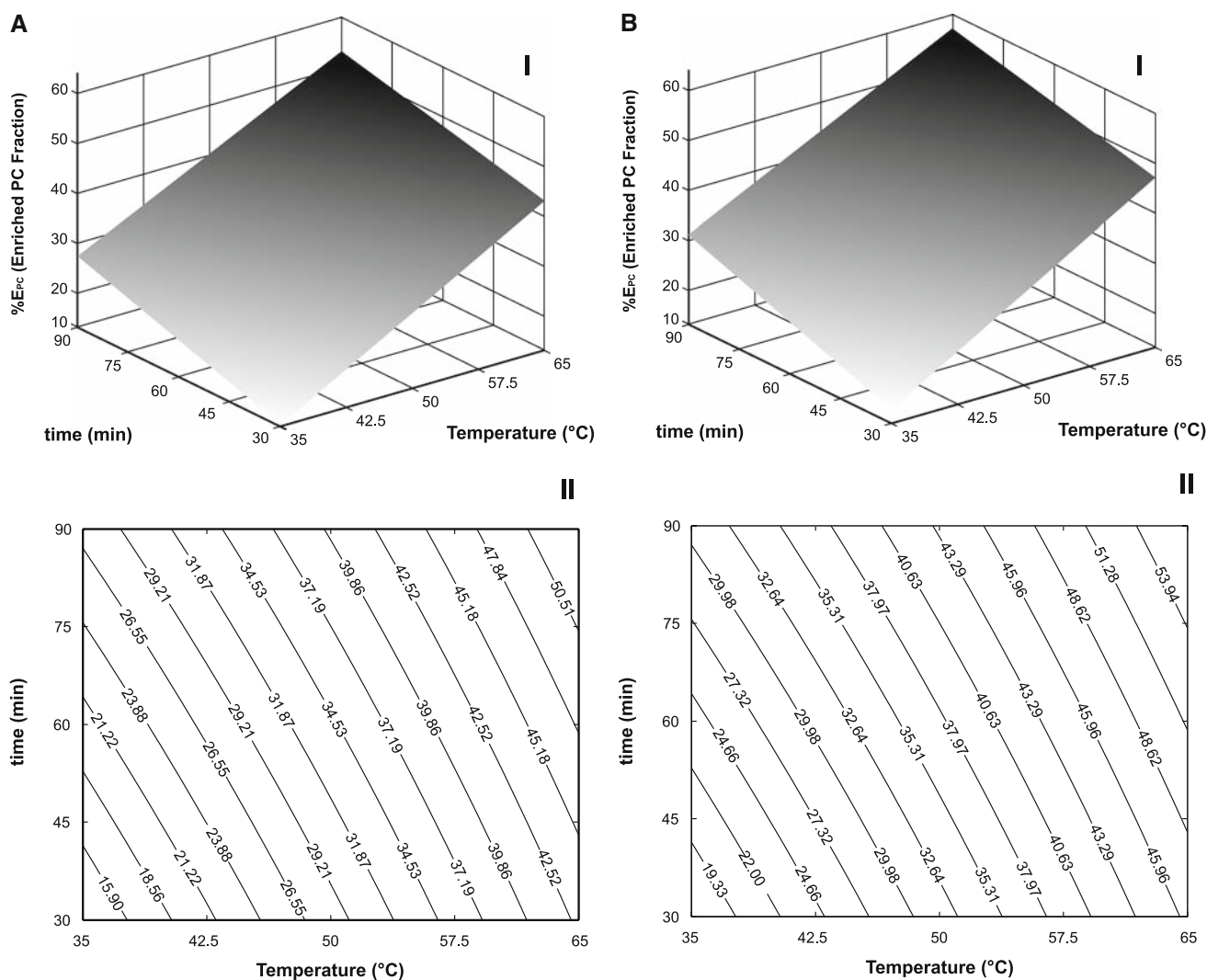
Fig. 5 Effect of temperature and incubation time on $\%E_{PC}$ in the PC enriched fraction with an ethanol/lecithin ratio (**a**) 2:1 and (**b**) 3:1. **I**: response surface curve and **II**: contour plot

best methodologies for analyzing phospholipids with high resolution and accuracy. It is applicable to vegetable sources such as soybean, corn and sunflower [24, 26]. ^{31}P NMR determinations of the different fractions demonstrated the high solubility of PC in absolute ethanol with an important enrichment in phosphatidylcholine (≈ 45–55%) with low PI contents ($<3\%$) for PC fractions in comparison with the phospholipid composition of the original sunflower lecithin (PC 16.2%, PI 16.5%, PE 5.3%, minor phospholipids 5.1%). In contrast, phosphatidylinositol changed from 28 to 34% in the corresponding PI enriched fractions according to its chemical structure and low solubility in ethanol (see Fig. 1) [5–7]. The results indicated that the operative conditions of this process did not markedly modify the phospholipid composition even though a slight diminution in PC content for the PC fraction at high temperature was noticed (Table 2).

The PC/PE ratio was relevant due to the potential industrial use of each fraction [7, 27]. PC and PI enriched fractions showed a mean PC/PE ratio of 8.7:1 and 1.6:1, respectively. These values showed that the two enriched fractions obtained were very different in terms of their phosphololipid composition and functionality in comparison with the original sunflower lecithin (PC/PE 3.0:1).

Taking into account enriched fraction yield% values and phospholipid composition, the percentage extraction coefficient ($\%E_{PC}$, $\%E_{PE}$, $\%E_{PI}$) values were determined as a function of the different processing conditions for each fraction (Fig. 3a–c). $\%E_{PC}$ values presented a highly significant increase ($p < 0.01$) for PC enriched fractions at increasing temperatures. The same trend was observed concerning incubation time (ranging from 30 to 60 min). However, the ethanol/lecithin ratios showed no relevant changes. With regard to $\%E_{PE}$, only the effect of temperature

was marked ($p < 0.05$). The above results showed the different distribution of the mixture of phospholipids in absolute ethanol, PC presenting a high solubility mainly at 65 °C. On the other hand, $\%E_{PI}$ exhibited low values ($<3\%$) under all the conditions assayed in the enrichment of PC fraction indicating a very low solubility of this compound in ethanol. The enriched PC fraction obtained at 65 °C, 90 min, ethanol-lecithin 3:1 could be the optimum one taking into account its enriched fraction yield and $\%E_{PC}$ from the initial sunflower lecithin. These results presented a good correlation with those obtained using soybean lecithin and a similar procedure [6, 13].

Taking into account the characteristics of the fractionation process, $\%E_{PC}$ was used as a tool for monitoring the evolution of this modification process. Then, a mathematical model was applied in order to explain the global influence of the parameters on the evolution of $\%E_{PC}$ in PC enriched fraction. The proposed model includes the variables and their interactions which presented significant differences ($p < 0.05$) at the corresponding ANOVA analysis Eq. 5.

$$\%E_{PC} = \%E_{PCmean} + 13.92 \times T + 6.05 \times t + 1.72 \\ \times \ EtOH/Lec - 0.95 \times t \times \ EtOH/Lec \quad (5)$$

where $\%E_{PC\ mean} = 35.87$; T: Temperature; t: time and EtOH/Lec: ethanol/lecithin ratio (normalized variables)

Figure 4 shows a good correlation between experimental and predicted values (mean percentage relative error = 5.1%, $R^2 = 0.98$), indicating a good fitting of the model. As shown in Eq. 5 the regression coefficient associated to temperature denotes the relevance of this parameter against the other variables studied. Also, the duration of the fractionation process presents a considerable contribution to PC separation from the original matrix towards the ethanol extract. Even though the ethanol/lecithin ratio and time—ethanol/lecithin ratio interaction were found to be significant ($p < 0.05$), a minor effect was produced on $\%E_{PC}$ levels. The interaction mentioned could be related to the ethanol availability recorded at a low ethanol/lecithin ratio (2:1).

Response surface curves and contour plots of $\%E_{PC}$ in the PC enriched fraction as a function of temperature (35–65 °C) and time of incubation (30–90 min) were obtained for an ethanol/lecithin ratio 2:1 (Fig. 5a-I and II) and 3:1 (Fig. 5b-I and II), respectively.

For both ethanol/lecithin ratios, a linear increase of $\%E_{PC}$ was observed after the fractionation process at increasing temperature and time of incubation. This behavior could be related with a minor incidence of the quadratic term in the mathematical model described. A low efficiency of PC extraction was evident for ethanol/lecithin ratio 2:1 (Fig. 5a-I and 5b-I).

The analysis of contour plots of $\%E_{PC}$ in the PC enriched fraction presented a mean variation of 12.1 units within the range of processing time at a constant temperature. On the other hand, more important changes were recorded (27.8 units) when only temperature was increased (Fig. 5a-II and 5b-II).

The application of the fractionation process to sunflower lecithin allowed us to obtain different enriched fractions on a laboratory scale. The study of the operating conditions on the fractionation process demonstrated a relevant influence of temperature, incubation time and to a minor extent of the ethanol/lecithin ratio on the enriched fraction yield% and selectivity of the main phospholipids (PC, PI) estimated by $\%E_{PL}$. Low solubility of PI in absolute ethanol was shown by the low values of $\%E_{PI}$ obtained for the PC enriched fractions. Changes of $\%E_{PC}$ values in PC fractions were recorded from 12.8 (35 °C, 30 min, 2:1) to 57.7 (65 °C, 90 min, 3:1). The highest levels of the processing variables studied could be considered the optimum conditions which lead to a major $\%E_{PC}$ value. Values of $\%E_{PC}$ and its mathematical modeling could be used as a marker for monitoring the evolution of the fractionation process.

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