Research Paper

Effect of processing parameters on sunflower phosphatidylcholine-enriched fractions extracted with aqueous ethanol

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Lecithins are widely used in the food industry because of their multifunctional characteristics. Fractionation of the original mixture of phospholipids in lecithin is desirable for certain applications. The influence of ethanol/water mixtures (90 : 10 to 96 : 4) and other operative conditions (temperature 35–65 °C, incubation time 30–90 min, solvent/lecithin ratio 2 : 1, 3 : 1) on the extraction of phosphatidylcholine (PC)-enriched fractions of sunflower lecithin (a non-GMO product) was investigated. Yield % and phospholipid composition of the enriched PC fractions as well as the residue were determined. The percent extraction coefficient of each phospholipid (E_{PC}, E_{PE} and E_{PI}) in the enriched PC fraction was calculated. Values of E_{PC} varied from 6.5 (35 °C, 30 min, 2 : 1, 90 : 10) to 52.6 (65 °C, 90 min, 3 : 1, 96 : 4). High temperature and long incubation time produced a significant increase of this coefficient ($p < 0.05$) while a high water content in the ethanolic mixture resulted in a considerable decrease in PC extraction. E_{PI} (<3%) values showed the high insolubility of phosphatidylinositol. Statistical analysis and response surface methodology evidenced the influence of the different variables on the extraction of PC-enriched fractions at laboratory scale.

Keywords: ^{31}P NMR / Aqueous ethanol fractionation / Phosphatidylcholine / Phospholipids / Sunflower lecithin

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1 Introduction

Lecithin is a mixture of phospholipids, mainly phosphatidylcholine (PC), phosphatidylethanolamine (PE) and phosphatidylinositol (PI), and other substances (glycolipids, complex sugars). It can be obtained from vegetable, animal and microbial sources. These days, vegetable lecithins (from soybean, rapeseed, sunflower) are obtained as a by-product of the oil refining process [1].

Native and modified lecithins are used in a wide range of industrial applications: nutritional, pharmaceutical applications, food, cosmetics, *etc*. [2, 3]. In the food industry, lecithin represents a multifunctional additive in the manufacture of chocolate, bakery and instant products, margarines, and mayonnaise, due to the characteristics of its phospholipids [4, 5].

Changes in the relative concentrations of the original phospholipid composition of lecithin due to the modification process (fractionation) can result in enriched fractions in certain phospholipids, with different physicochemical and functional properties which are also desirable for different industrial purposes [6–8].

Ethanol and aqueous alcoholic mixtures can be used for the fractionation of PC and PI, as other authors and patents have described. PC is relatively more soluble in ethanol than PI; thus, an ethanol extraction gives rise to a PC-enriched fraction [9–13]. This process can be carried out alone or in combination with other techniques such as chromatography as a further purification step.

Phospholipids contain lipophilic fatty acyl groups and hydrophilic headgroups; this amphiphilic structure makes them a good surface tension-reducing agent and thus a good emulsifier [14]. A PC-enriched fraction, due to its high PC/PE ratio and the lamellar phase structure of the PC at the oil-

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water interface, is believed to be a good oil-in-water (O/W) emulsifier [6, 15, 16].

In Argentina, the production of sunflower oils is of utmost importance, with the consequent economic relevance [17]. Sunflower lecithin might represent an alternative to soybean lecithin because it is considered a non-GMO product, which is in accordance with the preference of some consumers. Some authors have made contributions to the study of the physicochemical and functional properties of the native sunflower lecithin [18–22]. Nevertheless, the study of modification processes like fractionation has not been extensively applied to this type of lecithin.

It is important to consider that the ethanol fractionation process of lecithin may be affected by various factors (temperature, time of incubation, type of extraction solvent, extraction solvent/lecithin ratio) and their interactions [11, 23, 24]. In order to study the effect of the different factors on the desired response, the response surface method (RSM) was applied as an effective tool. RSM is a statistical-mathematical method based on quantitative data in an experimental design to determine and solve multivariable equations in order to optimize processes or products. RSM has become very popular for optimization studies in recent years [25, 26].

The aim of this work was to evaluate the influence of the main operating conditions on the application of a fractionation process to sunflower lecithin with aqueous ethanol at laboratory scale, in order to obtain different PC-enriched fractions as well as to optimize this process by using RSM.

2 Materials and methods

2.1 Materials

Sunflower lecithin was supplied by a local oil industry (Vicentin S.A.I.C.). 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.

All solvents used were of analytical grade.

2.2 Sunflower lecithin fractionation

The fractionation process was carried out on sunflower lecithin with the addition of different absolute ethanol/water mixtures (90 : 10 and 96 : 4) under different operative parameters such as temperature, time of incubation and extraction solvent/lecithin ratio (2 : 1, 3 : 1).

Samples of 30 g were incubated in a water bath in the range of 35–65 °C, 30–90 min with moderate agitation and then centrifuged at 1880 × g, 10 min, 10 °C. Afterwards, the corresponding ethanol extracts and residues were obtained and the absolute ethanol/water mixture was separated by evaporation under vacuum.

Aqueous ethanol-soluble and -insoluble phases (residues) were further deoiled with acetone, according to AOCS Official Method Ja 4-46, procedures 1–5 [27]. Then, the enriched PC and residual fractions, respectively, were obtained.

Both fractions were stored at 0 °C (Fig. 1). The fractionation procedure was performed in duplicate for each condition assayed.

The yield associated with each fraction was calculated according to:

$$\text{Enriched Fraction Yield}(\%) = \frac{\text{amount of fractionated sunflowerlecithin}}{\text{amount of starting sunflowerlecithin}} \cdot 100 \quad (1)$$

Also, the following equation must be considered:

$$\text{PC-enriched fraction yield (\%) + Residual fraction yield (\%) + \% Oil = 100\%} \quad (2)$$

2.3 Experimental design

The independent variables (temperature, time, ethanol/water ratio, extraction solvent/lecithin ratio) and their coded and uncoded levels used during the fractionation process on sunflower lecithin are shown in Table 1. These variables and their levels were selected according to a previous work carried out in our laboratory in order to achieve a successful optimization. The independent variables X_i were coded as x_i, which are defined as dimensionless according to Eq. (3):

$$x_i = (X_i - X_o)/\Delta X_i \quad (3)$$

where x_i is the coded value of an independent variable, X_i is the real value of an independent variable, X_0 is the real value of an independent variable at the center point, and ΔX_i is the step change value.

2.4 Phospholipid composition

Quantitative ^{31}P NMR analyses were carried out in a Bruker Avance 300 MHz automatic spectrometer using triphenylphosphate as internal standard (Spectral Service GmbH, Köln, Germany) [28–30].

The 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 was determined by this spectroscopic technique.

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

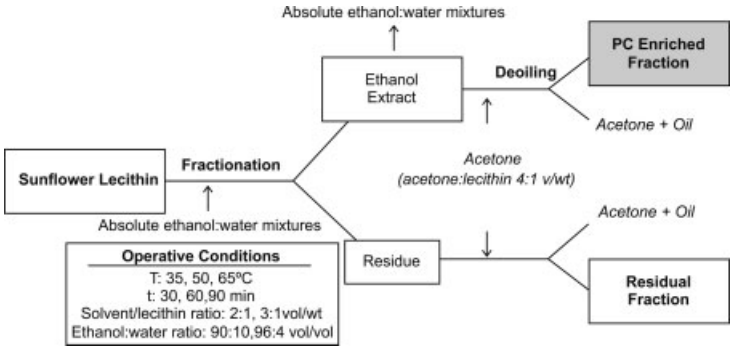

Figure 1. Flow diagram for the sunflower lecithin fractionation process with absolute ethanol/water mixtures.

Table 1. Independent variables and their levels used in the fractionation process of sunflower lecithin.

Independent variable	Uncoded symbol	Coded symbol	Variable value (coded value)		
Temperature [°C]	T	x_1	35 (–1)	50 (0)	65 (1)
Time [min]	t	x_2	30 (–1)	60 (0)	90 (1)
Absolute ethanol/water ratio [%]	EtOH/Water	x_3	90 (–1)	96 (0)	100 (1)[§]
Solvent/lecithin ratio [mL/g]	Solv/Lec	x_4	2 (–1)		3 (1)

[§] According to Cabezas *et al.* [24].

2.5 Data evaluation

The fractionation of each phospholipid for the different ethanol/water mixtures was monitored, and the corresponding extraction coefficients E_{PL} (E_{PC}, E_{PE} and E_{PI}) for PC-enriched fractions were calculated [24]. These values represent the percent contribution of each phospholipid in these fractions according to Eq. (4):

$$E_{PL}(\text{PC enriched fraction}) = \frac{m_{PL}(\text{PC enriched fraction})}{m_{PL}(\text{PC enriched fraction}) + m_{PL}(\text{Residual fraction})} \times 100 \quad (4)$$

where PL is PC, PE or PI; m_{PL} (PC-enriched fraction) = PC-enriched fraction yield % × % PL (PC-enriched fraction); and m_{PL} (Residual fraction) = Residual fraction yield % × % PL (Residual fraction).

The expression in Eq. (5) must be considered for calculations:

$$E_{PL}(\text{PC} - \text{enriched fraction}) + E_{PL}(\text{Residual fraction}) = 100\% \quad (5)$$

2.6 Statistical analysis

The experimental design used in the calculations was based on a full factorial design. Data were evaluated by analysis of variance (ANOVA). Significant variables and their interactions ($p < 0.01$) were used to fit the experimental data in the second-order polynomial equation and then obtain the coefficients of Eq. (6).

$$Y = \beta_o + \sum_{i=1}^{4} \beta_i x_i + \sum_{i=1}^{3} \sum_{j=i+1}^{4} \beta_{ij} x_i x_j \quad (6)$$

where Y is the response variable (E_{PC} in enriched PC fraction), x_i and x_j are the coded independent variables (see Table 1), and β_o, β_i, β_{ij} are the regression coefficients of variables for the intercept, linear and interaction regression terms, respectively.

The method of least squares was used to estimate the different parameters of Eq. (6). RSM was performed in order to optimize the fractionation process and obtain the maximum extraction coefficient E_{PC} in the enriched PC fraction [26, 31].

The statistical analyses were done using SYSTAT® 12 and response surface curves and contour plots were obtained using MATLAB® software.

3 Results

3.1 PC-enriched fraction yield

Deoiling of crude lecithin is a prerequisite for obtaining high-purity lecithin products like PC-enriched fractions. Acetone has been the solvent currently used in the industry for the separation of acylglycerols and PL [32]. Thus, the aqueous ethanol-soluble and -insoluble phases (residues) were deoiled with acetone according to Fig. 1.

The PC-enriched fraction yield % obtained as a function of the different assayed conditions is presented in Table 2. A significant increase ($p < 0.05$) of this parameter as a function

Table 2. Yield of PC fraction obtained by fractionation of sunflower lecithin under different operating conditions.

Sample	Coded independent variables[§]				PC-enriched fraction yield [%][$]	Residual fraction yield [%][#]
	x_1	x_2	x_3	x_4		
1	−1	−1	−1	−1	2.0	68.3
2	−1	−1	−1	1	2.4	67.9
3	−1	−1	0	−1	1.5	67.1
4	−1	−1	0	1	2.9	66.6
5[¥]	−1	−1	1	−1	3.4	62.8
6[¥]	−1	−1	1	1	4.7	61.6
7	−1	0	−1	−1	3.5	66.2
8	−1	0	−1	1	5.2	66.5
9	−1	0	0	−1	3.9	64.1
10	−1	0	0	1	5.9	63.7
11[¥]	−1	0	1	−1	5.8	60.7
12[¥]	−1	0	1	1	6.8	60.0
13	−1	1	−1	−1	5.0	65.0
14	−1	1	−1	1	6.2	63.7
15	−1	1	0	−1	6.2	61.9
16	−1	1	0	1	7.9	61.4
17[¥]	−1	1	1	−1	6.2	58.5
18[¥]	−1	1	1	1	8.5	57.7
19	0	−1	−1	−1	5.4	64.4
20	0	−1	−1	1	5.4	65.6
21	0	−1	0	−1	4.4	64.2
22	0	−1	0	1	4.4	65.9
23[¥]	0	−1	1	−1	7.7	58.8
24[¥]	0	−1	1	1	8.3	59.7
25	0	0	−1	−1	7.7	63.2
26	0	0	−1	1	7.7	64.0
27	0	0	0	−1	7.5	62.4
28	0	0	0	1	8.1	62.3
29[¥]	0	0	1	−1	10.5	56.7
30[¥]	0	0	1	1	9.7	57.1
31	0	1	−1	−1	8.5	62.2
32	0	1	−1	1	9.3	63.0
33	0	1	0	−1	9.2	60.2
34	0	1	0	1	10.7	59.7
35[¥]	0	1	1	−1	10.8	56.0
36[¥]	0	1	1	1	12.9	54.7
37	1	−1	−1	−1	5.8	64.4
38	1	−1	−1	1	7.9	63.1
39	1	−1	0	−1	9.1	60.0
40	1	−1	0	1	10.1	60.7
41[¥]	1	−1	1	−1	12.1	54.2
42[¥]	1	−1	1	1	14.0	53.2
43	1	0	−1	−1	8.6	62.1
44	1	0	−1	1	14.0	58.4
45	1	0	0	−1	10.6	59.0
46	1	0	0	1	14.4	56.1
47[¥]	1	0	1	−1	12.9	52.8
48[¥]	1	0	1	1	16.0	51.1
49	1	1	−1	−1	10.9	59.9
50	1	1	−1	1	13.6	57.6

Table 2. Continued.

Sample	Coded independent variables[§]				PC-enriched fraction yield [%][$]	Residual fraction yield [%][#]
	x_1	x_2	x_3	x_4		
51	1	1	0	–1	12.9	56.2
52	1	1	0	1	14.4	53.8
53[¥]	1	1	1	–1	13.4	53.0
54[¥]	1	1	1	1	16.9	50.2

[§] Coded symbols and levels of independent variables are according to Table 1.
[$] Values represent means (n = 2). The coefficient of variation (CV = 100 × mean/SD) was lower than 6%.
[#] Values represent means (n = 2). The coefficient of variation (CV = 100 × mean/SD) was lower than 5%.
[¥] Results according to Cabezas *et al.* [24].

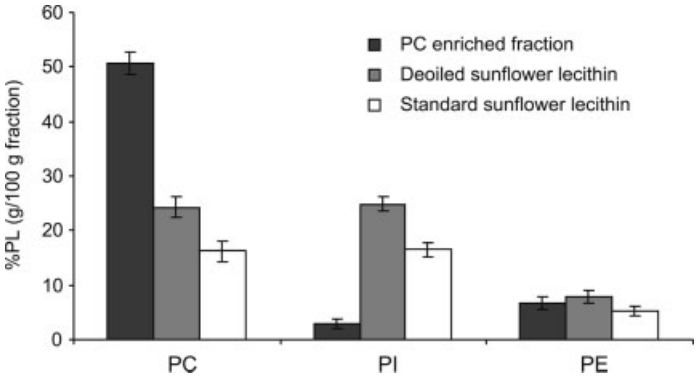

Figure 2. Phospholipid composition of the PC-enriched fraction, deoiled sunflower lecithin and standard sunflower lecithin. Error bars represent SD.

of the increase of temperature was recorded. A similar behavior was observed considering the time of extraction, especially between 30 and 60 min. These results presented a good correlation with those obtained in previous studies using absolute ethanol under similar experimental conditions for soybean and sunflower lecithins [24, 25]. On the one hand, the yield of the PC-enriched fractions decreased significantly ($p < 0.05$) with the increase in water content; on the other hand, an opposite behavior was obtained for the residual fraction, which is related to the principles of the fractionation process.

3.2 Phospholipid composition

The quantitative analysis of phospholipids was performed by ^{31}P NMR, 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 [6, 28]. The experimental information revealed that the different conditions studied did not show an important effect on the phospholipid composition of the modified lecithins. The results evidenced the high solubility of the phosphatidylcholine in the different extraction solvents assayed. The ^{31}P NMR determinations of the different enriched PC fractions exhibited an important concentration of PC (≈50.6 ± 2.1%) as well as low values of PI (<3 ± 0.8%) in comparison with the deoiled sunflower lecithin and the original sunflower lecithin assayed (Fig. 2).

3.2.1 PC/PE ratio

The PC-enriched fractions showed a mean PC/PE ratio of 7.6 : 1. This value evidenced that the enriched fractions obtained were very different in terms of their phospholipid composition and potential industrial use in comparison with the original sunflower lecithin (PC/PE 3.1 : 1). The PC/PE ratio of the PC-enriched fractions would favor their use as emulsifier agents in O/W emulsions instead of standard lecithin. Besides, fractions with PC/PE ratios over 4.2 play an interesting role as anti-spattering agents in the manufacture of margarine [6].

3.3 Percent extraction coefficients

Taking into account the yield values and composition of the enriched PC fractions, the percent extraction coefficients (E_{PC} and E_{PE}) of the PC-enriched fractions were determined and are shown in Table 3. The E_{PC} was analyzed by ANOVA to study the effect of the different operative conditions on the fractionation process of sunflower lecithin. The incidence of each variable was related to the percentage of variance explained (Table 4). Thus, E_{PC} presented a highly significant

Table 3. Percent extraction coefficient (E_{PC}, E_{PE}) of PC fractions obtained by fractionation of sunflower lecithin under different processing conditions.

Sample	Coded independent variables[§]				PC-enriched fraction	
	x_1	x_2	x_3	x_4	E_{PC}[$]	E_{PE}
1	–1	–1	–1	–1	6.5	2.7
2	–1	–1	–1	1	7.7	3.4
3	–1	–1	0	–1	5.1	1.6
4	–1	–1	0	1	9.0	3.1
5[¥]	–1	–1	1	–1	12.8	3.0
6[¥]	–1	–1	1	1	16.9	5.3
7	–1	0	–1	–1	11.9	3.9
8	–1	0	–1	1	18.2	6.5
9	–1	0	0	–1	17.8	4.3
10	–1	0	0	1	19.8	6.5
11[¥]	–1	0	1	–1	22.6	5.3
12[¥]	–1	0	1	1	24.5	5.6
13	–1	1	–1	–1	17.5	4.6
14	–1	1	–1	1	21.5	6.4
15	–1	1	0	–1	22.8	5.7
16	–1	1	0	1	28.7	8.5
17[¥]	–1	1	1	–1	23.7	5.6
18[¥]	–1	1	1	1	30.9	6.7
19	0	–1	–1	–1	18.7	5.4
20	0	–1	–1	1	16.2	6.5
21	0	–1	0	–1	14.5	5.2
22	0	–1	0	1	19.0	5.0
23[¥]	0	–1	1	–1	27.7	7.5
24[¥]	0	–1	1	1	28.0	8.0
25	0	0	–1	–1	25.4	6.9
26	0	0	–1	1	24.2	8.2
27	0	0	0	–1	25.2	12.2
28	0	0	0	1	26.1	8.3
29[¥]	0	0	1	–1	38.2	10.7
30[¥]	0	0	1	1	35.9	9.6
31	0	1	–1	–1	29.9	7.6
32	0	1	–1	1	29.2	10.1
33	0	1	0	–1	32.5	12.4
34	0	1	0	1	36.6	12.0
35[¥]	0	1	1	–1	40.3	10.3
36[¥]	0	1	1	1	45.8	12.2
37	1	–1	–1	–1	19.7	6.6
38	1	–1	–1	1	25.7	8.5
39	1	–1	0	–1	32.2	10.5
40	1	–1	0	1	32.8	11.4
41[¥]	1	–1	1	–1	44.5	13.8
42[¥]	1	–1	1	1	44.9	14.2
43	1	0	–1	–1	27.4	8.6
44	1	0	–1	1	33.3	15.8
45	1	0	0	–1	38.5	10.4
46	1	0	0	1	48.2	16.7
47[¥]	1	0	1	–1	48.6	14.5
48[¥]	1	0	1	1	53.7	17.2

Table 3. Continued.

Sample	Coded independent variables[§]				PC-enriched fraction	
	x_1	x_2	x_3	x_4	E_{PC}[$]	E_{PE}
49	1	1	−1	−1	36.7	11.7
50	1	1	−1	1	43.2	14.2
51	1	1	0	−1	47.2	15.1
52	1	1	0	1	52.6	18.1
53[¥]	1	1	1	−1	49.0	13.6
54[¥]	1	1	1	1	57.7	18.3

[§] Coded symbols and levels of independent variables are according to Table 1.
[$] Values represent means (n = 2). The coefficient of variation (CV = 100 × mean/SD) was lower than 7%.
[#] Values represent means (n = 2). The coefficient of variation (CV = 100 × mean/SD) was lower than 4%.
[¥] According to Cabezas *et al.* [24].

increase (p <0.01) as a function of increasing temperatures; this factor represents ≈54% of the variance of the process. A similar effect, though to a minor extent, was recorded for the time of incubation, especially between 30 and 60 min, and the ethanol/water mixtures. The E_{PC} values were considerably lower for the fractionation process with aqueous ethanol mixtures than those obtained with absolute alcohol. These results could be explained in terms of the extraction kinetics of PC, which is favored by the pure solvent. In addition, the solvent/lecithin ratio and the interaction temperature-ethanol/water mixtures did not produce an important change in the E_{PC} values.

On the other hand, the E_{PE} values varied between 3 and 18%, showing an increase as a function of temperature and time of process, particularly between 35 and 50 °C, 30 and 60 min, respectively (see Table 3). E_{PI} presented low values (<3%) under all conditions studied due to the insolubility of PI in the different ethanol/water mixtures (data not shown).

3.4 RSM

Taking into account the characteristics of the fractionation process, E_{PC} was used for monitoring its evolution. A mathematical model was applied in order to explain the global behavior of the different parameters and their interactions, which presented significant differences (p <0.01) in the corresponding ANOVA analysis (see Table 4). The regression coefficients, shown in Table 5, allowed the development of the following model:

$$E_{PC} = 28.60 + 11.43 \times T + 7.33 \times t + 6.30 \times \text{EtOH/water} + 1.73 \times \text{Solv/Lec} + 2.70 \times T\text{*EtOH/water} \quad (7)$$

where T is the temperature, t is the time, EtOH/water is the absolute ethanol/water ratio, and Solv/Lec is the solvent/lecithin ratio (coded values).

Table 4. Percentage of variance explained according to ANOVA in the fractionation process of sunflower lecithin under different processing conditions.

Variables[§]	Sum of squares	Percentage of variance explained
Significant variables[$]		
T	4864.940	54.0
t	1961.450	21.8
EtOH/Water	1518.878	16.9
Solv/Lec	162.240	1.8
T*EtOH/Water	244.165	2.7
Total model	*8751.673*	*97.2*
Non-significant variables		
T*t	4.352	0.0
T*Solv/Lec	46.563	0.5
t*EtOH/Water	57.904	0.7
t*Solv/Lec	22.948	0.3
EtOH/Water*Solv/Lec	3.554	0.0
Error	114.454	1.3
TOTAL	*9001.448*	*100*

[§] Uncoded symbols of independent variables according to Table 1.
[$] Variables and significant interactions of ANOVA (p <0.01).

The multiple coefficient of correlation (R = 0.958) and the total determination coefficient (R^2 = 0.918) indicate a good agreement between experimental and predicted values of E_{PC} (Fig. 3). The results obtained with this model presented an averaged absolute relative error (AARE%) = 14.32% (see Eq. 8), which also suggests an acceptable precision for the coefficient determination under the different operative conditions at laboratory scale.

$$AARE\% = \frac{100}{n} \cdot \sum \left| \frac{V\exp - Vpred}{V\exp} \right| \quad (8)$$

where V_{exp} is the experimental value and V_{pred} is the predicted value.

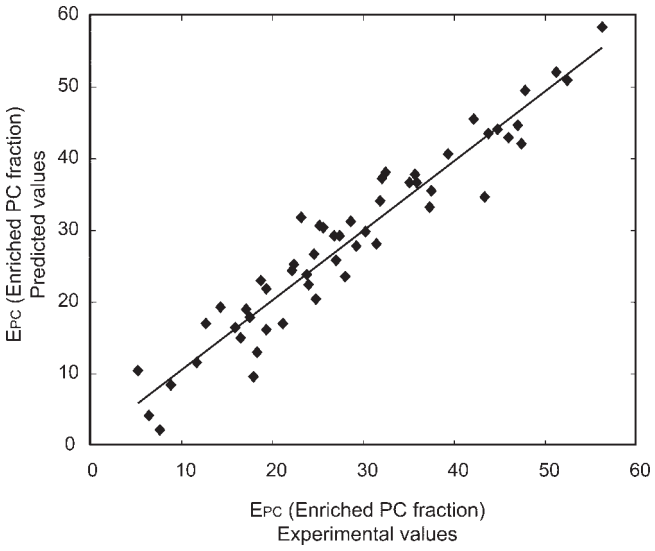

Figure 3. Correlation between predicted and experimental values of E_{PC} in the PC-enriched fraction obtained by the fractionation process of sunflower lecithin.

Table 5. Regression coefficients of the additive model for E_{PC} (PC-enriched fraction) obtained by fractionation of sunflower lecithin under different processing conditions.

Term	Regression coefficient[§]	Standard deviation
β_0	28.60	0.40
Linear		
β_1	11.43	0.49
β_2	7.33	0.40
β_3	6.30	0.48
β_4	1.73	0.49
Cross-product		
β_{14}	2.70	0.60

[§] Regression coefficients ($p < 0.01$).

Response surface curves (Fig. 4) and contour plots (Fig. 5) of E_{PC} as a function of temperature (35–65 °C), time of incubation (30–90 min) and ethanol/water mixtures (90 : 10, 96 : 4, 100 : 0) for both solvent/lecithin ratios (2 : 1 and 3 : 1) were obtained. These figures show that the highest levels of the processing variables could be considered the optimum conditions to achieve the highest E_{PC} value.

4 Conclusions

The application of the fractionation process on sunflower lecithin with absolute ethanol/water mixtures at laboratory scale allowed us to obtain fractions considerably enriched in PC. The effects of the operating conditions evidenced a strong influence of temperature and time of incubation on the extraction of PC from the original sunflower lecithin to the PC-enriched fraction. Also, the presence of water in the extraction solvent and the solvent/lecithin ratio are factors to be considered in the development and application of the fractionation process for optimizing the PC-enriched fraction yield. The highest levels of the processing variables studied could be considered the optimum conditions in order to lead to the highest E_{PC} values and achieve the best levels in terms of PC-enriched fraction yield. Further studies are necessary to evaluate the use of ethanol/water mixtures considering the cost of solvents and the enriched fraction yield for industrial purposes. The E_{PC} values and their mathematical modeling could be used as a good tool for monitoring the evolution of the fractionation process.

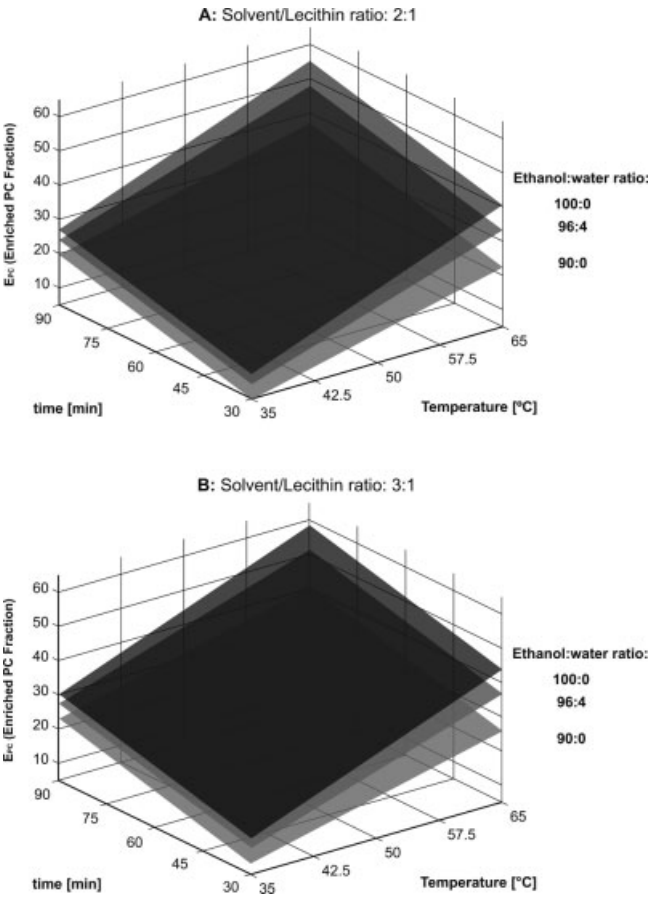

Figure 4. Response surface curves of E_{PC} obtained by absolute ethanol/water mixtures for different solvent/lecithin ratios: (A) 2 : 1 and (B) 3 : 1.

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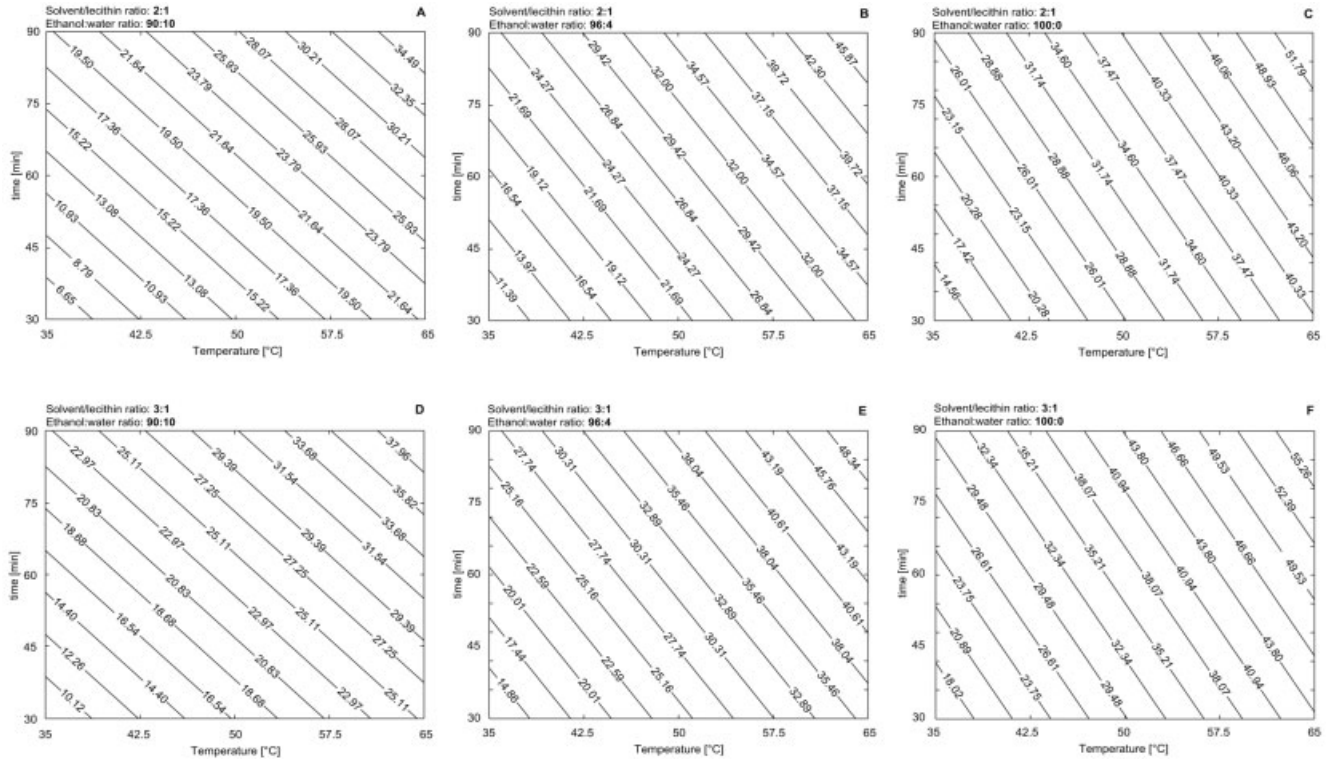

Figure 5. Contour plots of E_{PC} obtained by absolute ethanol/water mixtures for different solvent/lecithin ratios: (A) 2 : 1 and (B) 3 : 1.

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Conflict of interest statement

The authors have declared no conflict of interest.

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