Optimal Conditions for Fractionation of Rapeseed Lecithin with Alcohols

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Deoiled rapeseed lecithin was fractionated with ethanol, and optimum conditions have been determined to improve purified lecithin yield and phosphatidylcholine (PC) enrichment. The effect of extraction time, solvent volume, ethanol concentration and temperature on the yield and the PC enrichment have been described in the form of regression equations. A full factorial experiment method and a second-order orthogonal design were used in the study. The regression equations were calculated for the maximum value of the response functions optimized by an electronic data processing method, and the results (yield and PC enrichment calculated from regression equations) were compared with those obtained in control experiments. The use of calculated optimal parameters in the fractionation process led to 81–96% and 58% increments in yield and PC enrichment, respectively.

KEY WORDS: Alcohols, extreme experiments design, lecithin, lecithin fractionation, optimization of extraction process, phosphatidylcholine, rapeseed lecithin.

For many years, the pharmaceutical and food industries have taken a keen interest in vegetable lecithins (1). The quality of purified lecithin and the field of its application significantly depend on plant source and processing conditions. Our previous studies (2,3) showed that purified rapeseed lecithin of high quality could be prepared directly from the lecithin wet gum obtained from double-zero rapeseed varieties (low in erucic acid and glucosinolates).

It is well known that fractionation of soya lecithin with alcohols depends on several extraction conditions, including extraction time, solvent volume, mixing intensity, solvent polarity and temperature (3–5). Optimization of extraction parameters by conventional methods is arduous and time-consuming. Recently, interest in statistical experimental designs has been observed, especially in optimizing parameters in chemical processes and in the pharmaceutical industry (6–11).

We have determined the optimum conditions of deoiled rapeseed lecithin fractionation with ethanol by means of mathematical optimization methods to improve purified lecithin yield and phosphatidylcholine (PC) enrichment.

MATERIALS AND METHODS

The deoiled rapeseed lecithin from double-zero varieties low in erucic acid and glucosinolates was prepared by deoiling with acetone and dehydration of the wet gums as described previously (3). The lecithin contained 29.1% phosphatidylcholine (Ja 7b-91) and 98% acetone (Ja 4-4b) insolubles (American Oil Chemists' Society methods). The chemicals of analytical grade were supplied by POCh (Gliwice, Poland), the phospholipid standards were from Sigma (St. Louis, MO), the hexane and isopropanol of high-performance liquid chromatography-grade (HPLC) were from Merck (Darmstadt, Germany).

Lecithin fractionation. Deoiled rapeseed lecithin (30 g) and an appropriate volume of alcohol were introduced into a flask fitted with a stirrer and reflux condenser and placed in an ultrathermostat. The extraction process was carried out for parameters described in the experimental designs. The final mixtures were filtered through a sintered-glass funnel under water aspirator vacuum, and the ethanolic extracts were evaporated and dried under vacuum (40°C, 5–10 mm Hg) to constant weight. PC content of the fractionated lecithins was determined by HPLC.

PC content determination. The lecithin analysis was accomplished with a HPP 5001 HPLC system (Laboratorni Pristroje, Praha, Czechoslovakia) fitted with a 150 × 3.3 mm analytical column packed with 10 μm Separon SG X C-18 (Tessek, Praha, Czechoslovakia). The separated components were quantitatively measured by a refractometric detector (in an ultrathermostatic system) with an integrator. Samples were injected in the same solvent (20 μL of 1% wt/vol solution) used for elution. Phospholipids were separated by isocratic elution with hexane/isopropanol/water in ratio 1:4:1 (vol/vol/vol). Column flow rate was 0.5 mL/min and the column temperature was 30°C.

RESULTS AND DISCUSSION

Initially, we determined the effect of extraction time (t), solvent volume (V), ethanol concentration (C) and temperature (T) on the yield and the quality of the final lecithin. The effects of these parameters on the lecithin yield and the content of PC are presented in Figure 1.

This preliminary study on the fractionation process was carried out to define a range of variables necessary in design of an experimental matrix.

The effect of time on fractionations carried out at 20°C, 95% EtOH in water and a solvent/lecithin ratio of 5 L/kg are shown in Part A of Figure 1. PC content increased from 29.1% (deoiled lecithin) to 49% during the first 5 min of extraction, then decreased to 40% after 15 min. Simultaneously, yields of fractionated lecithin increased during the first 5 min, then leveled off. Thus, short extraction times favor both yield and PC content.

Part B of Figure 1 shows the effect of solvent/lecithin ratio (L/kg) on yield and PC content at an extraction time of 15 min, a temperature of 20°C and 95% ethanol in water. Although yields increased with larger solvent/lecithin ratios, PC content decreased at higher solvent/lecithin ratios. Thus, for the optimization studies, values in the 5–10 L/kg were selected.

The water content in ethanol also has a significant effect on a lecithin quality and extraction yield in the fractionation process (Part C, Fig. 1). During this process, the water content of about 15–20% vol/vol leads to lecithin agglutination. Thus, for the design matrix, ethanol concentrations in the range of 92–98% vol/vol were used.

Upon the basis of apparent changes in extraction yields and PC contents with temperature (Part D, Fig. 1), as well

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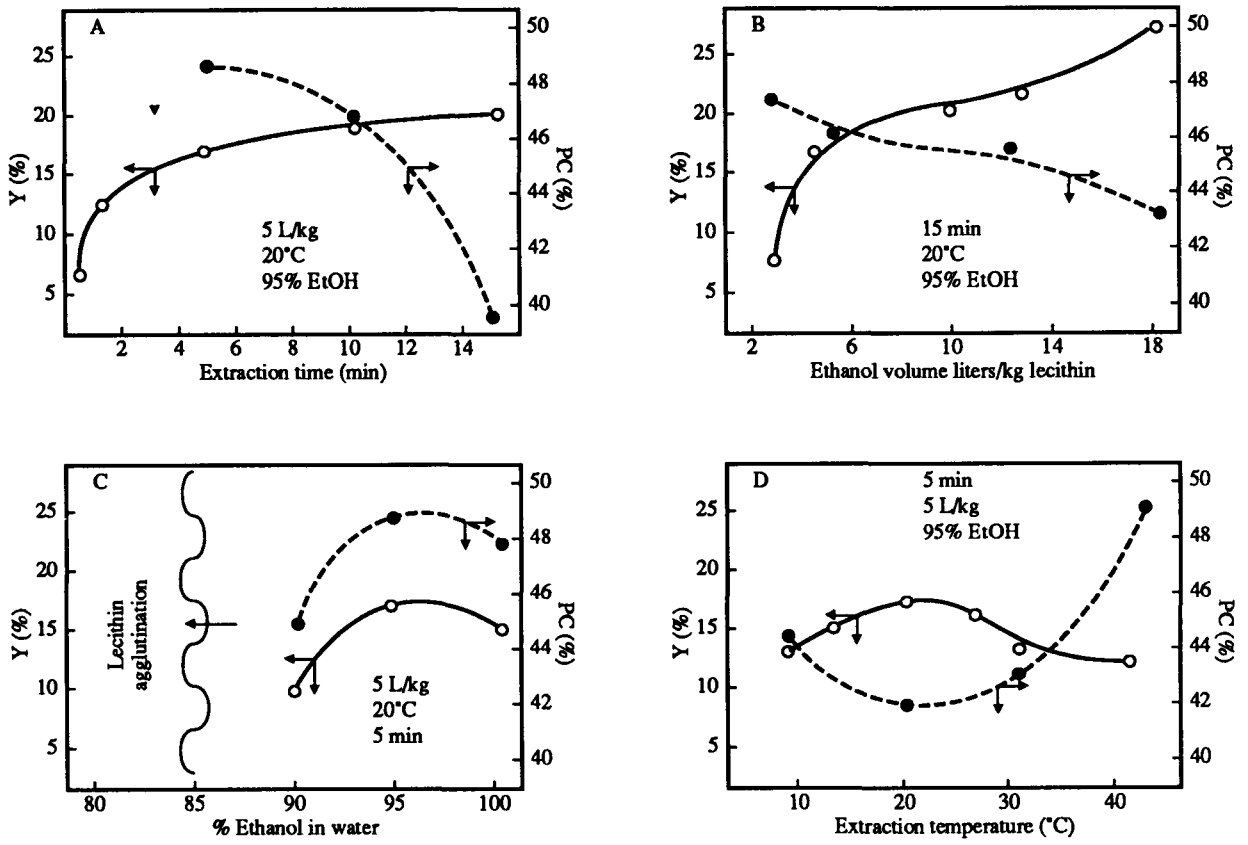

FIG. 1. Effect of extraction time (Part A), solvent volume (Part B), ethanol concentration in water (Part C) and temperature (Part D) on phosphatidylcholine content (PC•) and extraction yield (Y∘).

as taking into account the cost of energy, the range of 15–25°C was accepted for the optimization study in spite of the increment of PC content with temperature (the yields are approximately constant).

It is not clear from the results of preliminary experiments whether the optimum conditions for the process operation would be within the investigated range of variables. For that reason, the full factorial experimental design was applied at first (design 2^4, a combination of four variables on two levels, sixteen experiments). The values of parameters used in the experiments are given in Table 1. The resulting data were next calculated to extract yields (ratio of lecithin amount obtained to raw material used) and PC enrichment (E):

$$E\% = \frac{\%PC \text{ in extract} \cdot \text{extract amount [g]}}{\%\ PC \text{ in raw lecithin} \cdot \text{raw lecithin amount [g]}} \cdot 100\% \quad [1]$$

According to the design matrix (Table 2) for the experiments carried out, the yields obtained were from 13.0 to 27.6% (Table 3) while the PC content varied in the range of 38.5 to 48.5%. Taking the yields and PC contents into consideration, the calculated PC enrichment ranged between 18.8 and 43.8. To simplify the calculation of regression coefficients, the real values of the variables (z_j) used in the experiments were standardized to become dimensionless values (x_j): +1 for the higher level, −1 for the lower level and 0 for the basic one (z_j^0) from the formula:

$$x_j = \frac{z_j - z_j^0}{\Delta z_j} \quad [2]$$

These standardized values were used in the calculation of regression equation coefficients by the least-square method. The regression coefficients were calculated from the standard equation, which in a matrix notation has a form:

TABLE 1

Real Values and Code Symbols of the Variables Used in the Optimization Procedure[a]

Variables (z_j)	t (min)	V (L/kg)	C (%)	T (°C)
Code symbols	x_1	x_2	x_3	x_4
Basic level (z_j^0)	10	7.5	95	20
Interval of variation (Δz_j)	5	2.5	3	5
Higher level (+1)	15	10.0	98	25
Lower level (−1)	5	5.0	92	15

[a]t, extraction time; V, solvent volume; C, ethanol concentration; T, temperature of extraction.

TABLE 2

Design Matrix of the Full Factorial Experiment (regression Equation 4)

Experiment number	Levels of the code symbols of variables															
	x_6	x_1	x_2	x_3	x_4	x_1x_2	x_1x_3	x_1x_4	x_2x_3	x_2x_4	x_3x_4	$x_1x_2x_3$	$x_2x_3x_4$	$x_1x_2x_4$	$x_1x_3x_4$	$x_1x_2x_3x_4$
1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2	+	−	−	+	+	+	−	−	−	−	+	+	−	+	−	+
3	+	+	−	−	+	−	−	+	+	−	−	+	+	−	−	+
4	+	−	+	−	+	−	+	−	−	+	−	+	−	−	+	+
5	+	+	−	+	−	−	+	−	−	+	−	−	+	+	−	+
6	+	−	+	+	−	−	−	+	+	−	−	−	−	+	+	+
7	+	+	+	−	−	+	−	−	−	−	+	−	+	−	+	+
8	+	−	−	−	−	+	+	+	+	+	+	−	−	−	−	+
9	+	+	−	+	+	−	+	+	−	−	+	−	−	−	+	−
10	+	−	+	+	+	−	−	−	+	+	+	−	+	−	−	−
11	+	+	+	−	+	+	−	+	−	+	−	−	−	+	−	−
12	+	−	−	−	+	+	+	−	+	−	−	−	+	+	+	−
13	+	+	+	+	−	+	+	−	+	−	−	+	−	−	−	−
14	+	−	−	+	−	+	−	+	−	+	−	+	+	−	+	−
15	+	+	−	−	−	−	−	−	+	+	+	+	−	+	+	−
16	+	−	+	−	−	−	+	+	−	−	+	+	+	+	−	−

TABLE 3

Yields and Phosphatidylcholine (PC) Enrichment Obtained for Rapeseed Lecithin Fractionation

Experiment number	Response functions		
	FFE[a]		SOE[b]
	Yield	PC enrichment	yield
1	27.6	43.8	27.6
2	16.6	27.2	16.6
3	15.4	23.6	15.4
4	17.4	26.2	17.4
5	17.0	27.8	17.0
6	19.0	30.2	19.0
7	17.4	25.2	17.4
8	12.6	18.8	12.6
9	18.6	28.8	18.6
10	22.4	36.8	22.4
11	21.4	33.4	21.4
12	14.0	21.0	14.0
13	24.0	38.0	24.0
14	15.6	23.6	15.6
15	13.0	20.2	13.0
16	14.4	22.6	14.4
17	—	—	22.6
18	—	—	23.4
19	—	—	20.6
20	—	—	22.6
21	—	—	13.4
22	—	—	20.6
23	—	—	15.6
24	—	—	21.0
25	—	—	17.6

[a]Full factorial experiment.
[b]Second-order orthogonal experiment.

$$X^TXB = X^TY \qquad [3]$$

where X = the matrix of independent variables (Table 2); Y = the column vector of results (yields, PC enrichment) (Table 3); B = the column vector of regression coefficients; and X^T = transposition of an X-matrix. Incorporation into the regression equation of a dummy variable $x_0 = +1$ gives a set of standard equations with as many equations as the number of unknown coefficients (number of experiments).

The variances of the response functions (yield, PC enrichment) were determined based on the additional three complementary trials carried out for the basic level of parameters. For the extract yields and for the corresponding PC enrichment, the resulting variances were $\sigma_0^2 = 0.90$ and $\sigma_0^2 = 1.120$, respectively.

The results obtained in the experiments were subsequently calculated to multidimensional regression equations with monomials describing the interdependent effect of variables (*e.g.*, $b_{uj}x_ux_j$) in general form:

$$y = b_0x_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_4x_4 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{14}x_1x_4 + b_{23}x_2x_3 + b_{24}x_2x_4 + b_{34}x_3x_4 + b_{123}x_1x_2x_3 + b_{234}x_2x_3x_4 + b_{124}x_1x_2x_4 + b_{134}x_1x_3x_4 + b_{1234}x_1x_2x_3x_4 \qquad [4]$$

Based on well-known formulas (12), the regression equation coefficients and their statistical significance (Student's *t*-test) were calculated as stated in Table 4. All regression coefficients obtained for full factorial experiments are calculated with the same accuracy:

$$\sigma_{bj}^2 = \frac{\sigma_0^2}{n} \qquad \text{(see Table 4)} \qquad [5]$$

where n is a number of experiments and σ_0^2 is a variance of the response function. When the error estimation value obtained from the formula

$$t_b = \frac{|\text{coeff. value}|}{\sigma_{bj}} \qquad [6]$$

is lower than the critical value $t_p(f) = 4.3$ from a table of Student's distribution for significance level $P = 0.05$ and degree of freedom $f = 2$, the respective regression coefficient is statistically insignificant.

Taking the significant coefficients of the full factorial experiment into consideration, the regression equation, correlation coefficient (R) and F-Fisher value (F) for the extraction yield take the form:

$$y = 17.900 + 1.400x_1 + 2.550x_2 + 2.200x_3 + 1.275x_4 + 0.750x_1x_2 + 0.600x_2x_3 + 0.475x_2x_4 \qquad [7]$$

where $R = 0.995$; $F_{cal.} = 3.13 < F_{(8,2)} = 19.37$; and for the PC enrichment:

TABLE 4

Coefficients of the Regression Equations of the Full Factorial Experiment[a]

Symbol of regression coefficient	Response functions: Yield			PC enrichment		
	Value	Error estim.[b]	Signif. coeff.[c]	Value	Error estim.[d]	Signif. coeff.[c]
b_0	17.900	59.7	17.900	27.950	26.4	27.950
b_1	1.400	18.7	1.400	2.150	8.1	2.150
b_2	2.550	34.0	2.550	4.075	15.4	4.075
b_3	2.220	29.6	2.220	4.075	15.4	4.075
b_4	1.275	17.0	1.275	2.150	8.1	2.150
b_{12}	0.750	10.0	0.750	0.925	3.5	—
b_{13}	0.300	4.0	—	0.425	1.6	—
b_{14}	0.175	2.3	—	0.150	0.6	—
b_{23}	0.600	8.0	0.600	1.100	4.2	—
b_{24}	0.475	6.3	0.475	0.875	3.3	—
b_{34}	−0.075	1.0	—	−0.025	0.1	—
b_{123}	0.100	1.3	—	0.200	0.8	—
b_{234}	0.075	1.0	—	0.100	0.4	—
b_{124}	−0.025	0.3	—	0.325	1.2	—
b_{134}	0.075	1.0	—	−0.575	2.2	—
b_{1234}	−0.025	0.3	—	−0.100	0.4	—

[a]PC, phosphatidylcholine.
[b]Calculated for standard deviation of coefficients $\sigma_{bj} = 0.075$.
[c]Critical value $t_{0.05}$ (2) = 4.3.
[d]Calculated for standard deviation of coefficients $\sigma_{bj} = 0.265$.

TABLE 5

Design Matrix of the Second-Order Orthogonal Experiment (regression Equation 9)

Experiment number	Levels of the code symbols of variables													
	x_0	x_1	x_2	x_3	x_4	x_1'	x_2'	x_3'	x_4'	x_1x_2	x_1x_3	x_1x_4	x_2x_3	x_2x_4	x_3x_4
1	+	+	+	+	+	0.2	0.2	0.2	0.2	+	+	+	+	+	+
2	+	−	−	+	+	0.2	0.2	0.2	0.2	+	−	−	−	−	+
3	+	+	−	−	+	0.2	0.2	0.2	0.2	−	−	+	+	−	−
4	+	−	+	−	+	0.2	0.2	0.2	0.2	−	+	−	−	+	−
5	+	+	−	+	−	0.2	0.2	0.2	0.2	−	+	−	−	+	−
6	+	−	+	+	−	0.2	0.2	0.2	0.2	−	−	+	+	−	−
7	+	+	+	−	−	0.2	0.2	0.2	0.2	−	−	−	−	−	+
8	+	−	−	−	−	0.2	0.2	0.2	0.2	+	+	+	+	+	+
9	+	+	−	+	+	0.2	0.2	0.2	0.2	−	+	+	−	−	+
10	+	−	+	+	+	0.2	0.2	0.2	0.2	−	−	−	+	+	+
11	+	+	+	−	+	0.2	0.2	0.2	0.2	+	−	+	−	+	−
12	+	−	−	−	+	0.2	0.2	0.2	0.2	+	+	−	+	−	−
13	+	+	+	+	−	0.2	0.2	0.2	0.2	+	+	−	+	−	−
14	+	−	−	+	−	0.2	0.2	0.2	0.2	+	−	+	−	+	−
15	+	+	−	−	−	0.2	0.2	0.2	0.2	−	−	−	+	+	+
16	+	−	+	−	−	0.2	0.2	0.2	0.2	−	+	+	−	−	+
17	+	0	0	0	0	−0.8	−0.8	−0.8	−0.8	0	0	0	0	0	0
18	+	1.414	0	0	0	1.2	−0.8	−0.8	−0.8	0	0	0	0	0	0
19	+	−1.414	0	0	0	1.2	−0.8	−0.8	−0.8	0	0	0	0	0	0
20	+	0	1.414	0	0	−0.8	1.2	−0.8	−0.8	0	0	0	0	0	0
21	+	0	−1.414	0	0	−0.8	1.2	−0.8	−0.8	0	0	0	0	0	0
22	+	0	0	1.414	0	−0.8	−0.8	1.2	−0.8	0	0	0	0	0	0
23	+	0	0	−1.414	0	−0.8	−0.8	1.2	−0.8	0	0	0	0	0	0
24	+	0	0	0	1.414	−0.8	−0.8	−0.8	1.2	0	0	0	0	0	0
25	+	0	0	0	−1.414	−0.8	−0.8	−0.8	1.2	0	0	0	0	0	0

$$y = 27.950 + 2.150x_1 + 4.075x_2 + 4.075x_3 + 2.150x_4 \quad [8]$$

where $R = 0.961$; $F_{cal.} = 4.19 < F_{(11,2)} = 19.41$. The evaluation of Equations 7 and 8 by F-Fisher test shows the statistical significance of those equations. The high values of correlation coefficients confirm the proper description of the examined process and the influence of selected parameters on yield and PC enrichment. In Equation 7, all the first-degree monomials (main effect) and some of the second-degree values (interdependent effect of variables) are significant. Thus it could be suggested that the response function (the yields) describes the process examined near the quasi-stationary surface (near the extremum). Based on this, the next experiments were carried out for the second-order orthogonal design. To save the orthogonality of the matrix, in the case of four variables for this design, it was necessary to carry out 25 experiments (sixteen for the standard matrix, eight for star points and one for the central point) (Table 5). The experimental results were applied to the multi-

TABLE 6

Coefficients of the Regression Equation of the Second-Order Orthogonal Experiment (extraction yield)

Symbol of regression coefficient	Value	Error estimation[a]	Significant coefficients[b]
b_0	18.544	61.8	18.544
b_1	1.330	19.9	1.330
b_2	2.680	40.0	2.680
b_3	2.120	31.6	2.120
b_4	1.260	18.8	1.260
b_{11}	0.420	4.0	—
b_{22}	−1.585	15.0	−1.585
b_{33}	−1.530	14.4	−1.530
b_{44}	−0.930	8.8	−0.930
b_{12}	0.750	10.0	0.750
b_{13}	0.300	4.0	—
b_{14}	0.175	2.3	—
b_{23}	0.600	8.0	0.600
b_{24}	0.475	6.3	0.475
b_{34}	−0.075	1.0	—

[a]Calc. for standard deviation of coefficient groups $\sigma_{bj} = 0.067$, $\sigma_{buj} = 0.075$, $\sigma_{bjj} = 0.106$.
[b]Critical value $t_{0.05}\,(2) = 4.3$.

dimensional quadratic regression equation in general form:

$$y = b_0x_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_4x_4 + b_{11}x_1' + b_{22}x_2' + b_{33}x_3' + b_{44}x_4' + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{14}x_1x_4 + b_{23}x_2x_3 + b_{24}x_2x_4 + b_{34}x_3x_4 \quad [9]$$

where $x_i' = x_i^2 - 0.8$. The regression coefficients obtained for the second-order orthogonal design (Table 6) are calculated with different accuracy in contrast to the full factorial design:

$$\frac{\sigma_0^2}{\sum_{i=1}^{n} x_{ij}^2}$$

In this case, the coefficients of the second-degree monomials are highly significant, which confirms that the response function describes the process near the extreme (the quasi-stationary surface). The normalized regression equation obtained for the second-order design is:

$$y = 21.780 + 1.330x_1 + 2.680x_2 + 2.120x_3 + 2.160x_4 + 0.750x_1x_4 + 0.600x_2x_3 + 0.475x_2x_4 - 1.585x_2^2 - 1.530x_3^2 - 0.930x_4^2 \quad [10]$$

where $R = 0.978$; $F_{cal.} = 6.24 < F_{(14,2)} = 19.42$. All regression equations in the normalized form were transformed into standard forms (real value) for the computation. For the extraction yield, the two equations were obtained:

$$y = -0.11635 - 0.17000t - 7.94000V + 0.13333C - 0.03000T + 0.06000tV + 0.08000VC + 0.03800VT \quad [11]$$

(the full factorial experiment) and (the second-order orthogonal experiment):

$$y = -1557.28860 - 0.18400t - 4.08400V + 32.40667C + 1.45500T + 0.06000tV + 0.08000VC + 0.03800VT - 0.25360V^2 - 0.17000C^2 - 0.03720T^2 \quad [12]$$

PC enrichment can be described by the regression equation with first-degree monomials only:

$$y = -126.21635 + 0.043000t + 1.63000V + 1.35833C + 0.43000T \quad [13]$$

Subsequently, the regression equations were optimized for the maximum value of the response functions by an electronic data processing method. The computation was performed by a mini-computer IBM 386 based (Samsung, Taiwan) on the standard computer program Eureka to search for the maximum value of the response functions with the gradient method and the iterative one near the extreme (for restrictive values of parameters see Table 7). Optimal values of extraction time, solvent volume, alcohol

TABLE 7

Rapeseed Lecithin Extraction Yields and Phosphatidylcholine (PC) Enrichment Calculated and Obtained in Control Experiments

Parameters	FFA[a] Yield	FFA[a] PC enrichment	SOE[b] yield
Restrictive values for computation			
Regression equation	[11]	[13]	[12]
Response function (%)	25–90	32–90	40–90
Extraction time (min)	5–10	5–10	5–60
Solvent volume (L/kg)	3–33	3–33	3–33
Alcohol concentration (%)	90–100	90–100	90–100
Temperature (°C)	10–30	10–30	10–70
Optimal values			
Extraction time (min)	10	10	60
Solvent volume (L/kg)	22.9	30.3	13.7
Alcohol concentration (%)	100	100	98
Temperature (°C)	30	30	23
Results			
Response function (%):			
calculated from equation	52	76	54
experimental	54	69	50
Difference (% of calculation)	3.8	9.2	7.4
Maximum values obtained in primary experiments	27.6	43.8	27.6

[a]Full factorial experiment.
[b]Second-order orthogonal experiment.

concentration and temperature of extraction are listed in Table 7.

We also carried out some control experiments for the optimal parameters calculated. The results are also reported in Table 7. A comparison of the yield and PC enrichment values calculated and from experiments (for optimal parameters) showed differences below 10%. Taking the complexity of the natural lecithin used in the experiments into consideration, the resulting differences are small. The use of calculated optimal parameters in fractionation of deoiled rapeseed lecithin leads to an 81–96% increment in yield and a 58% increase in PC enrichment.

Our results show that highest yields can be obtained with anhydrous ethanol at a temperature of about 30°C. The remaining parameters investigated, *i.e.*, extraction time and solvent volume, are probably related to each other. The maximum value of the extraction yield and the PC enrichment could be obtained in the short time of about 10 min with a large amount of solvent (lecithin to ethanol ratio, 1:30). Then, the sixfold longer extraction time leads to about 50% reduction in the solvent volume for the maximum yield and PC enrichment.

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