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ORIGINAL PAPER

Enrichment of Lysophosphatidylcholine in Canola Lysolecithin

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Abstract Lysophosphatidylcholine (LPC) possesses excellent oil-in-water emulsifying properties and health benefits. The objective of this study was to produce an LPC-enriched fraction from lysolecithin generated during enzymatic degumming of crude canola oil. Three alcohols (methanol, ethanol and isopropanol) were evaluated for their effectiveness at enriching LPC. A 3 × 3 full factorial design was employed to study the effects of two processing parameters (temperature and alcohol/lysolecithin ratio) on three responses (yield and LPC concentration of alcohol soluble fraction, and LPC recovery) with the most effective alcohol. Ethanol was found to be the best solvent to enrich LPC in lysolecithin. An ethanol soluble fraction with more than 50 % LPC was produced. Quadratic models with $R^2 > 0.9$ were developed to describe the relationship between the processing parameters and the responses in the 3 × 3 full factorial experiment. Both ethanol soluble fraction yield and LPC recovery increased with increasing temperature and ethanol/lysolecithin ratio. LPC concentration in the ethanol soluble fraction was enhanced with decreasing temperature and ethanol/lysolecithin ratio. According to the analysis, ethanol soluble fractions with LPC concentration higher than 66 % could be obtained at temperatures of 0–40 °C and an ethanol/lysolecithin ratio of 2:1 (v/w).

Keywords Lysophosphatidylcholine · Lysolecithin · Fractionation · Optimization

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Introduction

Lysophosphatidylcholine (LPC) is a class of lysophospholipids (LPL) containing one glycerol unit, one acyl chain and a phosphate group to which a choline molecule is attached [1]. Although it is only present in small amounts in most tissues, LPC plays important roles in many physiological processes such as reproduction, vascular development and the nervous system [2–4]. LPC can alter the permeability of cell membranes, and enhance the absorption of other nutrients [5–7]. LPC is now included in many bodybuilding supplements to aid the absorption of other ingredients. LPC has anti-inflammatory properties, and is protective against lethal sepsis [8]. LPC also possesses excellent emulsifying properties owing to its amphiphilic nature. It is the desired emulsifier for oil-in-water emulsions in food, cosmetics and pharmaceutical products. LPC is an excellent starting material for synthesis of phosphatidylcholine (PC) with desired fatty acid composition [9, 10].

Since LPC is only a minor component of biological cell membranes, it does not occur abundantly in nature. Usually, LPC is either prepared from glycerol phosphorylcholine (GPC) by esterification with fatty acids or from PC by enzymatic hydrolysis, which is followed by separation of LPC from other substances in the reaction mixture. In either way, the starting materials GPC and PC are generally first prepared from lecithin, a mixture of different phospholipids which is obtained from the degumming of vegetable oils [1]. The application of phospholipases in commercial vegetable oil degumming has been expanding. Degumming of vegetable oils with phospholipase A_1 or A_2 in particular, has presented a new form of by-product, lysolecithin, which has not been exploited to its full capacity for value-added product development. The lysolecithin from degumming with phospholipase A_1 or A_2 is expected to contain

an appreciable amount of lysophospholipids which are the hydrolysates of phospholipids. Since PC is one of the major phospholipids in vegetable oils, it is expected that lysolecithin from vegetable oils would contain substantial amounts of LPC. So far, no attempt has been made to recover lysophosphatidylcholine from lysolecithin, which is inexpensive and readily available.

The objective of this study was to obtain a lysophosphatidylcholine enriched fraction from lysolecithin which was recovered from gum produced during enzymatic degumming of crude canola oil.

Materials and Methods

Materials

Crude canola oil was provided by ADM (Decatur, IL, USA). Phospholipase A1, Lecitase Ultra, was from Novozymes (Bagsvaerd, Denmark). This enzyme has a declared activity of 10,000 LU/g (Lipase Unit/gram). One LU is defined as the amount of enzyme that releases 1 μmol of titratable butyric acid from tributyrin substrate in 1 min at 40 °C at pH 7. Lysophosphatidylcholine standard was purchased from Avanti (Alabaster, Alabama). Acetone, methanol, ethanol, and isopropanol (ACS grade) were from Pharmco-AAPER (Brookfield, CT, USA). Chloroform and methanol (HPLC grade) were from EMD Millipore (Cincinnati, OH, USA).

Recovery of Canola Lysolecithin

Canola lysolecithin was recovered from crude canola oil by enzymatic degumming with Lecitase Ultra. Crude canola oil, 1,500 g, was placed in a 2-L jacketed glass reactor, which was connected to a refrigerated/heated circulating bath (Model 11679, VWR, Bristol, CT, USA). The oil was heated to 80 °C and 1.95 g 50 % (wt%) citric acid solution was added. The oil and citric acid mixture was homogenized using an Omni homogenizer (GLH, Kennesaw, GA, USA) with a 20 mm × 195 mm saw tooth generator probe at 24,000 rpm for 1 min. The resulting mixture was stirred at 80 °C and 500 rpm for 20 min using an overhead stirrer (Eurostar, IKA, Wilmington, NC, USA). After cooling the mixture to 50 °C, 1.90 g of 4 N NaOH solution was added into the oil and citric acid mixture followed by the addition of 0.06 g of enzyme (40 ppm in the oil) and 34.9 g of deionized water. The total amount of water used was 2.5 % based on the weight of oil. The mixture was homogenized at 24,000 rpm for 1 min, and then stirred at 50 °C and 500 rpm for 6 h. After the enzymatic reaction, the temperature of the mixture was increased to 80 °C and kept at this temperature for 0.5 h to deactivate the enzyme. The mixture was then centrifuged using a floor type centrifuge (Sorvall RC 5C, Thermo, Asheville, NC, USA) at 3,000 rpm for 5 min to separate the wet gum from the degummed oil. Lysolecithin was prepared by washing the wet gum with cold acetone according to AOCS official method Ja 4-46 [11] and was kept at −20 °C until further use and analysis.

Solvent Selection for Enriching LPC

Methanol, ethanol, and isopropanol (IPA) were evaluated for their efficiency at producing fractions with a high concentration of LPC from canola lysolecithin. Canola lysolecithin, 2.5 g, was mixed with 15 mL solvent and mixed with a magnetic stirrer (Spin Master 4802, Cole-Parmer, Vernon Hills, IL, USA) at 50 °C for 0.5 h. The mixture was centrifuged at 5,000 rpm for 5 min. Solvent soluble and insoluble fractions were separated and dried under a vacuum at 80 °C. Dried fractions were weighed and stored at −20 °C until further analyses.

Optimization of LPC Enrichment

A 3 × 3 full factorial design was employed to optimize LPC enrichment with the most effective solvent. Two processing parameters, temperature (0, 30 and 60 °C) and ethanol/lysolecithin ratio (2, 6 and 10 mL/g) were studied as the independent variables. The dependent responses were solvent soluble fraction yield, LPC concentration of solvent soluble fraction and LPC recovery. All the other conditions used in the factorial experiments were the same as those employed in section "Solvent Selection for Enriching LPC". LPC recovery was calculated by the following formula:

$$\text{LPC recovery (\%)} = \frac{\text{Amount of LPC in solvent soluble fraction}}{\text{Amount of LPC in starting material}} \times 100\,\%.$$

LPC Analysis

LPC content of canola lecithin samples were determined by HPLC-ELSD using the method reported by Sugawara and Miyazawa (1999) with modification. The HPLC system was an Alliance 2695 (Waters Corp., Milford, MA). The Evaporative Light Scattering Detector (ELSD) was an Alltech 2000 (ALL Tech Associates Inc., Deerfield, IL). The conditions for ELSD were a nitrogen flow rate of 3.5 L/min, impactor ON, and a drift tube temperature of 80 °C. A μPorasil silica column (125 Å, 10 μm, 300 mm × 3.9 mm id, Waters, Milford, MA, USA) was used. The mobile phase consisted of chloroform (A) and 95:5 (v/v) methanol/water (B). The gradients used for elution of

the compounds were 99:1 A/B at 0 min to 75:25 A/B at 15 min, 75/25 A/B to 10:90 A/B at 20 min, 10:90 A/B held constant to 25 min, and 10:90 A/B to 99:1 A/B at 30 min. The column was kept at 30 °C, and the mobile phase flow rate was 1 mL/min. The samples were dissolved in 2:1 (v/v) chloroform/methanol to achieve a concentration of 20 mg/mL and filtered through a 0.45 μm syringe filter prior to injection. Sample injection volume was 20 μL. An external standard curve was constructed to quantify LPC in samples.

Statistical Analysis

All experiments and analytical tests were carried out at least in duplicate. For the one-way Analysis of Variance (ANOVA) method means were compared using Fisher's Least Significant Difference method after ANOVA *F* test showed significance. ANOVA was performed using SAS 9.3 (SAS Institute Inc., Cary, NC, USA). All statistical tests were performed at a significance level of 0.05.

Results and Discussion

The phosphorus content in crude canola oil was reduced from 485 ppm to 5 ppm by the enzymatic degumming method used in this study. The canola lysolecithin collected from enzymatic degumming contained 19.5 % LPC, which is much higher than the LPC levels in lecithin obtained from water degumming (<6 %) [12]. The high level of LPC in the lysolecithin is due to the hydrolysis of PC by the phospholipase A_1 used for enzymatic degumming. This result shows that lysolecithin from enzymatic degumming is a rich source of LPC.

Effect of Solvent Type on LPC Enrichment

The effect of solvent type on fraction yields and LPC concentration in the fractions is shown in Table 1. Fractionation of canola lysolecithin with methanol resulted in the highest amount of solvent soluble fraction, which was about 32 % of the starting material (Table 1). Ethanol gave a significantly ($P < 0.05$) lower soluble fraction yield (17.1 %) than methanol. IPA produced the lowest amount of soluble fraction (8.2 %). Accordingly, the highest amount of solvent insoluble fraction was produced by IPA, which was followed by ethanol and methanol. LPC was enriched in the solvent soluble fractions regardless of the solvent type. Methanol soluble fraction had the lowest LPC concentration (28.9 %), indicating the poor selectivity of methanol for LPC. IPA showed a better selectivity for LPC than methanol. It produced a solvent soluble fraction containing 40.9 % of LPC, which was more than two-fold enrichment from the original lysolecithin. Ethanol exhibited the highest selectivity toward LPC among the three alcohols examined. The concentration of LPC in ethanol soluble fraction was 53.2 %, which was almost three times as high as that in the original canola lysolecithin. Ethanol is the most effective solvent for enrichment of PC from lecithin produced by water degumming [13]. It is not surprising that this alcohol also shows excellent selectivity for LPC, which has only one less fatty acid than PC in its chemical structure. Among the solvent insoluble fractions, IPA insoluble fraction contained the highest amount of LPC (15.8 %), which was followed by methanol (13.7 %) and ethanol insoluble (12.3 %) fractions. To the best of our knowledge this is the first study to evaluate different solvents for LPC enrichment from canola lysolecithin obtained from enzymatic degumming. In summary ethanol was more effective than methanol and IPA in enriching LPC in the solvent soluble fraction. For this reason, ethanol was chosen as the solvent to optimize the LPC enrichment process through a factorial design.

Optimization of LPC Enrichment

Based on the minimum amount of solvent required to suspend the lysolecithin sample, the 2:1 ethanol/lysolecithin ratio was chosen as the lowest level of this factor. The choice for the highest level of ethanol/lysolecithin ratio (10:1) was based on the result for PC enrichment from lecithin by ethanol [13]. The range for temperature was chosen according to the capacity of the equipment

Table 1 Effect of solvent type on yields and LPC concentration of fractions

Solvent	Soluble fraction		Insoluble fraction	
	Fraction yield (wt%)	LPC concentration (wt%)	Fraction yield (wt%)	LPC concentration (wt%)
Methanol	31.9 ± 0.6^{a}	28.9 ± 0.4^{c}	69.3 ± 0.9^{a}	13.7 ± 0.3^{b}
Ethanol	17.1 ± 0.7^{b}	53.2 ± 1.0^{a}	82.7 ± 0.3^{b}	12.3 ± 0.4^{a}
IPA	8.2 ± 0.2^{c}	40.9 ± 0.4^{b}	91.7 ± 1.4^{c}	15.8 ± 0.2^{c}

Values are means ± SEM

Means in the same column with the same superscript letter are not significantly different from each other ($P > 0.05$)

Table 2 Yield of soluble fraction, LPC concentration of soluble fraction and LPC recovery obtained from the full factorial experiment

Run	Temperature (°C)	Ethanol/lysolecithin ratio (mL/g)	Ethanol soluble fraction yield (%)	LPC concentration (%)	LPC recovery (%)
1	0	2	3.3 ± 0.1	67.6 ± 0.2	11.4 ± 0.4
2	0	6	4.9 ± 0.1	59.3 ± 0.2	15.3 ± 0.2
3	0	10	5.9 ± 0.1	58.4 ± 0.1	20.1 ± 0.5
4	30	2	6.4 ± 0.2	65.3 ± 1.4	21.5 ± 0.2
5	30	6	13.7 ± 0.1	61.3 ± 0.3	43.2 ± 0.2
6	30	10	16.3 ± 0.1	59.4 ± 0.2	49.6 ± 0.2
7	60	2	7.1 ± 0.1	61.8 ± 1.4	22.4 ± 0.4
8	60	6	19.5 ± 0.1	55.1 ± 0.5	50.1 ± 0.5
9	60	10	25.1 ± 0.1	50.9 ± 0.8	65.7 ± 1.0

Values are means ± SEM, $n = 2$

Table 3 Estimated coefficients of the quadratic models

Soluble fraction yield			LPC concentration of soluble fraction			LPC recovery		
Variable	Parameter estimate	*P* value	Variable	Parameter estimate	*P* value	Variable	Parameter estimate	*P* value
Intercept	−0.28	0.7748	Intercept	70.89	<0.0001	Intercept	−1.11	0.7365
Temp	0.10	0.0106	Temp	0.13	0.0438	Temp	0.53	0.0005
Ratio	1.72	0.0003	Ratio	−2.51	0.0012	Ratio	5.73	0.0003
Temp × ratio	0.032	<0.0001	Temp × ratio	−0.003	0.4685	Temp × ratio	0.072	<0.0001
Temp × ratio	−0.001	0.0133	Temp × temp	−0.003	0.0014	Temp × temp	−0.007	0.0007
Ratio × ratio	−0.12	0.0009	Ratio × ratio	0.13	0.0206	Ratio × ratio	−0.38	0.0012

$P < 0.05$ indicates statistical significance

available to this study and the literature [14]. The experimental data obtained from the 3 × 3 full factorial experiment is shown in Table 2. The yields of ethanol soluble fraction ranged from 3.3 to 25.1 %. The highest yield of alcohol soluble fraction was obtained at temperature of 60 °C and ethanol/lysolecithin ratio of 10:1; while the lowest yield was obtained at temperature of 0 °C and ethanol/lysolecithin ratio of 2:1. The LPC concentration in ethanol soluble fractions was between 50.9 and 67.6 %. The conditions for the highest and lowest LPC concentrations were temperature of 0 °C and ethanol/lysolecithin ratio of 2:1, and temperature of 60 °C and ethanol/lysolecithin ratio of 10:1, respectively. Under the conditions investigated in the full factorial experiments, 11.4–65.7 % of LPC was extracted from canola lysolecithin. The highest and lowest LPC recovery was obtained at a temperature of 60 °C and ethanol/lysolecithin ratio of 10 and at 0 °C and ethanol/lysolecithin ratio of 2:1, respectively.

The following quadratic models were developed to fit the experimental data, and describe the relationship between the independent variables (temperature and ethanol/lysolecithin ratio) and the dependent variables (ethanol soluble fraction yield, LPC concentration of ethanol soluble fraction and LPC recovery).

$$\text{Y-ES}\,(\%) = -0.28 + 0.1\,T + 1.72\,R - 0.001\,T^2 - 0.12\,R^2 + 0.0032\,R \times T \quad (1)$$

$$\text{LPC-C}\,(\%) = 70.89 + 0.13\,T - 2.51\,R - 0.003\,T^2 + 0.13\,R^2 - 0.003\,R \times T \quad (2)$$

$$\text{Y-LPC}\,(\%) = -1.11 + 0.53\,T + 5.73\,R - 0.007\,T^2 - 0.38\,R^2 + 0.072\,R \times T. \quad (3)$$

Y-ES, LPC-C and Y-LPC represent the estimated values for ethanol soluble fraction yield, LPC concentration in ethanol soluble fraction and LPC recovery, respectively. T and R represent temperature and ethanol/lysolecithin ratio, respectively. All quadratic models shown above were significant ($P < 0.05$). The R^2 of all quadratic models were greater than 0.9, indicating that these models explained more than 90 % of the variation in the experimental data. As shown in Table 3, the linear and quadratic terms of temperature and ethanol/lysolecithin ratio had significant effects on all three responses. A significant ($P < 0.05$) interaction between temperature and ethanol/lysolecithin ratio was observed for the ethanol soluble fraction yield and LPC recovery, but not for the LPC concentration of the ethanol

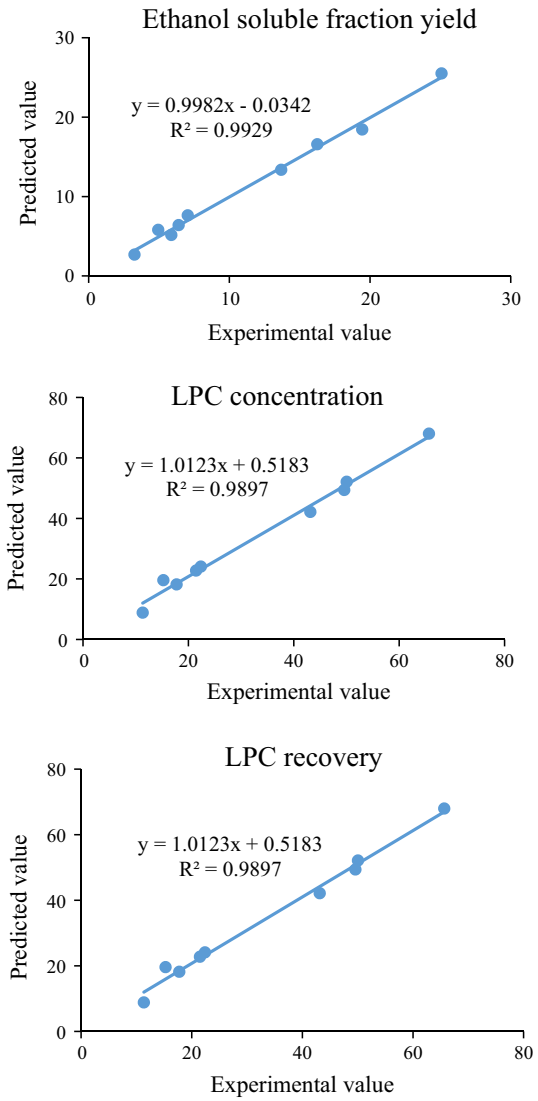

Fig. 1 Correlation between experimental values and the values predicted by the quadratic models

soluble fraction. The values predicted by these quadratic models are in good agreement with the experimental data (Fig. 1), which demonstrates the adequacy of the models to explain the relationship between the independent variables and the responses within the ranges investigated in this study.

Figure 2 shows the effects of temperature and the ethanol/lysolecithin ratio on the yield and LPC concentration of the ethanol soluble fraction, and LPC recovery. At a temperature of 0 °C, the ethanol soluble fraction yield was increased only slightly with an increase in the ethanol/lysolecithin ratio. As the temperature increased, the improvement in the soluble fraction yield became more pronounced with the increase in the ethanol/lysolecithin ratio. LPC concentration in the ethanol soluble fraction decreased with increasing temperature and ethanol/lysolecithin ratio. The way by which LPC recovery was affected by temperature and ethanol/lysolecithin ratio was similar to that for the ethanol soluble extraction yield.

Within the ranges investigated in this study, the analysis showed that the highest ethanol soluble fraction yield (>24 %) and LPC recovery (>65 %) were obtained at temperature of 60 °C and an ethanol/lysolecithin ratio of 10. LPC concentrations higher than 66 % were obtained between temperatures of 0 and 40 °C and an ethanol/lysolecithin ratio of 2:1. According to the analysis, temperatures higher than 60 °C and ethanol/lysolecithin ratios higher than 10:1 tend to result in a higher amount of the ethanol soluble fraction and thus higher LPC recovery; while temperatures lower than 0 °C and ethanol/lysolecithin ratios lower than 2:1 show the tendency to produce an ethanol soluble fraction with an even higher LPC concentration. In order to improve LPC concentration, a further study focusing on the processing conditions with temperatures and ethanol/lysolecithin ratios lower than 0 °C and 2:1, respectively, seems reasonable. However, it is not economic due to the low ethanol soluble fraction yield (<3 %) and thus low LPC recovery (<15 %). Based on the experimental data and the analysis, a temperature of 40 °C and an ethanol/lysolecithin ratio of 2:1 is considered to be the optimal condition for LPC enrichment, where a compromise between LPC concentration (>66 %) and ethanol soluble fraction yield (about 25 %) can be achieved.

Conclusion

Regardless of the type of solvent used, LPC was enriched in solvent soluble fractions of canola lysolecithin. Ethanol was more effective than methanol and IPA in enriching LPC. A 3 × 3 full factorial design was employed to optimize LPC enrichment by ethanol extraction at temperatures of 0–60 °C and ethanol/lysolecithin ratios between 2:1 and 10:1. Quadratic models were established for predicting the ethanol soluble fraction yield, LPC concentration in the ethanol soluble fraction and LPC recovery. Within the ranges investigated, an ethanol soluble fraction with the highest LPC concentration (>66 %) was obtained at temperatures between 0 and 40 °C and an ethanol/lysolecithin ratio of 2:1. According to the analysis temperatures and ethanol/lysolecithin ratios lower than 0 °C and 2:1, respectively, may produce fractions with LPC concentrations higher than those obtained in this study. However, no further study was conducted under these conditions due to a low ethanol soluble fraction and LPC

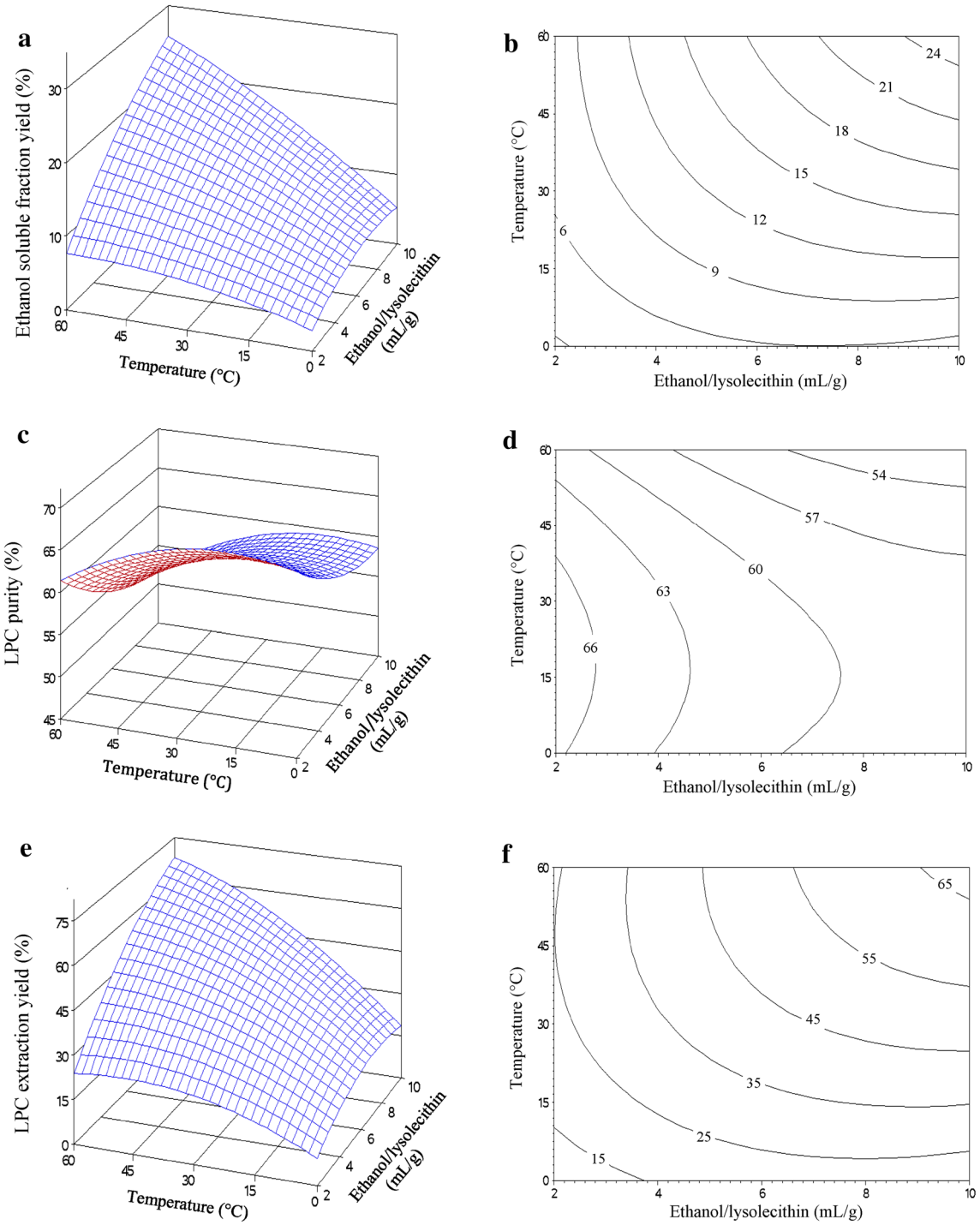

Fig. 2 Response surface and contour plots for the ethanol soluble fraction yield, LPC concentration of the ethanol soluble fraction and LPC recovery from the full factorial experiment. **a** and **b** are response surface and contour plots for the ethanol soluble fraction yield, respectively; **c** and **d** are response surface and contour plots for LPC concentration of the ethanol soluble fraction, respectively; **e** and **f** are response surface and contour plots for LPC recovery, respectively

recovery. From an economic point of view, a temperature of 40 °C and an ethanol/lysolecithin ratio of 2:1 appear to be better conditions to achieve a LPC-enriched fraction from lysolecithin.

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