Enzymatic extraction and functional properties of phosphatidylcholine from chicken liver

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ABSTRACT An environmentally sustainable method to extract phosphatidylcholine (**PC**) from chicken liver (**PCCL**) and its functional properties were studied. The extraction times, enzymatic hydrolysis time, the solid-liquid ratio as well as types of enzymes (protamex proteinase and neutral proteinase) were investigated. Furthermore, the content of PCCL, emulsifying properties and solubilities of PCCL were also determined. The optimum conditions of extracting PCCL were found to be: reaction time of 3.75 h, enzymatic hydrolysis time of 85.22 min, 1: 3.15 (w/v) of solid-liquid ratio, using protamex proteinase, and the yield and concentration of PCCL was 88.92% and 0.89 mg/mL, respectively. Solubility and emulsifying properties of PCCL showed that the HLB value of PCCL was 10, and in ethanol and glycerol, the solubility of PCCL was 0.5850 g/mL and 0.0965 g/mL, respectively, which was shown to have good ethanol solubility and lipophilicity. From the perspective of green production and high-value utilization of by-products, PCCL could be used as a potential new lecithin source, providing ideas for the development and application of PC of animal origin.

Key words: phosphatidylcholine, enzymatic extraction, solubility, emulsifying properties, by-products

2022 Poultry Science 101:101689
https://doi.org/10.1016/j.psj.2021.101689

INTRODUCTION

Phosphatidylcholine (**PC**) is called "the third nutrient", which widely exists in animals and plants. It has a certain content in egg yolk, beans, animal liver, fish (especially head), brain, bone marrow, heart, kidney, lung, milk, sesame, edible fungi, yam, agaric, corn, sunflower, flax, yeast, and other foods (Garba et al., 2020; Ciji et al., 2021). In addition, it is an important source of phosphorus, essential fatty acids and choline needed by the human body (Topuz et al., 2021), because of its pleiotropic effect on health, people have a great interest in PC (Huang et al., 2012). However, consumer concerns about genetically modified soybeans and allergies to soy products (Li and Guo, 2016), which requires exploring alternative commercial sources of PC. The main components of phospholipids in animal liver are PC, phosphatidylinositol (**PI**) and phosphatidylethanolamine (**PE**), accounting for about 80% of the total phospholipids in liver, and the content is similar to that of soybean phospholipids. Chicken liver is one of the main by-products after slaughtering, accounting for 2.0 to 2.5% of the body weight. In 2020, the global broiler production will increase to 100.827 million tons (Zhang et al., 2021), assuming that the slaughtering rate of chickens was 65%, the annual production of chicken liver in the world is as high as 70 million tons (Xiong et al., 2017). However, its special fishy smell is difficult to be accepted by people. If the by-products of chicken liver are converted and reused, it will produce objective economic benefits and reduce the environmental pollution caused by the by-products, therefore, it is considered to extract phosphatidylcholine from chicken liver (**PCCL**) as a new commercial source of lecithin.

Organic solvent extraction is a common method for extracting PC, such as rainbow trout fish (Guo et al., 2022), egg yolk, and hemerocallis citrina Baroni. (Topuz et al., 2021). It has the advantages of short production cycle, large production capacity, and high yield. Kovalcuks and Duma (2016) found that 97.89% PC and 99.81% PE were dissolved in ethanol. Chen et al. (2019) optimized the extraction process of PC from egg yolk powder by ethanol, and the content of PC reached 75.59%. Luz and Wang (2005) extracted PC with ethanol and precipitated with acetone, and the purity of PC was more than 95%. However, very little research on the extraction process of PC from chicken liver has been conducted. Proteolytic enzymes show great application prospect in food, biological science, and medical industry because of their wide substrate specificity, wide range of

Received July 15, 2021.
Accepted December 8, 2021.

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pH activity and etc. (Wang et al., 2017; Li et al., 2018). Therefore, Arroyo et al. (2017) used proteinase (trypsin, neutral proteinase, etc.) to hydrolyze large molecule protein into small molecule peptide, making the PC coated with protein dissolved in the solvent fully. It can overcome the shortcomings of traditional organic solvent extraction method, such as residual solvents in the final product, environmental pollution, difficulty in solvent regeneration, and so on (Djas and Henczka, 2018; Haq et al., 2021), obtaining high-quality PC from chicken liver under a mild operating environment (Huang et al., 2020).

Improper alkali disposal or improper design of waste control system often leads to soil pollution, and these environmental changes will have a substantial adverse impact on human life (Vindula et al., 2020; Di Giglio et al., 2021). Accordingly, we selected proteinase without additional adjustment of pH, such as neutral proteinase and protamex proteinase, to extract PCCL by enzymatic hydrolysis-assisted organic solvent extraction. In summary, the goal of the research was 1) establishing an environmentally friendly method to extract and recover PCCL and 2) analyzing the functional properties of the PCCL to provide a theoretical basis for its application.

MATERIALS AND METHODS

Materials

Fresh chicken liver obtained from Yurun Food Co., Ltd (Jiangsu Province, China) were used to extract the PCCL. Soybean PC and egg PC were purchased from Zhejiang Yinuo Biological Technology Co., Ltd (Ningbo, Zhejiang Province, China) and Shenzhen Lefu Biological Technology Co., Ltd (Shenzhen Province, China). Protamex proteinase (120 U/mg) and neutral proteinase (100 U/mg) were purchased from Yuanye Biological Co., Ltd. (Shanghai, China). All reagents were of analytical grade unless otherwise described.

Preparation of PCCL

Refer to Wang's method and make some changes (Wang et al., 2014). Dispersing the chicken liver in distilled water by the homogenizer, then inactivating the enzymes in chicken liver by hyperthermia. According to the preliminary experimental results, the ultrasonic wave was applied with an output power of 200 W (on-time 2 s, off-time 3 s), and the process lasted for 10 min (Shi et al., 2020). The neutral proteinase and protamex proteinase were added into the suspension and stirred, after the enzymatic hydrolysis, they were inactivated in boiling water for 20 min. Next, 95% ethanol with different solid-liquid ratio was added into the suspension. Finally, the mixture was extracted at 35°C for different time, repeating the above steps several times until the filter residue becomes colorless. The filtrate was evaporated by rotary evaporator, and the oil was collected by washing the rotary flask with petroleum ether. Extracting and purification the total lipids were conducted by Bligh/Dyer methods (Bligh and Dyer, 1959).

Experimental Conditions of Single Factor Experimental

In this study, the effects of enzymatic hydrolysis time, extraction time and material-liquid ratio were researched on the yield and extraction rate of PCCL. The hydrolysis time is 30, 60, 90, and 120 min, the chicken liver to 95% ethanol ratio is 1/2 (V/V), 1/3 (V/V), 1/4 (V/V), and the extraction time is 2, 3, 4, 5, and 6 h, respectively.

Experimental Design of Response Surface Methodology

According to the single factor experimental results, we chose the condition with the highest extraction rate of PC (4 h extraction reaction, chicken liver to 95% ethanol ratio was 1/3 (V/V), 90 min enzymatic hydrolysis) for response surface analysis. In accordance with this result, the Box-Behnken experimental design of the yield with the three process parameters and three coded levels for each parameter are shown in Table 1, where the ranges of Factors A, B, and C are further reduced.

Verification Experiment of Response Surface Methodology

The response surface methodology (**RSM**) was used to obtain the optimal conditions, and 3 groups of parallel experiments were carried out to verify the optimal solution.

Qualitative Analysis of PCCL

Preparation of standards and sample: standard solutions and PCCL sample solutions (10 mg/mL) were prepared by dissolving 0.1 g of PC standard and PCCL sample in 10 mL of chloroform-methanol (3: 2, V/V), respectively.

Thin layer chromatography (**TLC**) conditions: A self-made silica gel G plate was prepared, and the loading amount of standard and PCCL sample were 2 μL and 4 μL, respectively. The standard and PCCL sample were applied onto the analytical TLC plate and developed using the mobile phase of chloroform: carbinol: water (65: 25: 4, V/V/V). They were placed in the

Table 1. Box-Behnken experimental design with three process parameters and three coded levels for each parameter.

	Factor		
Level	A Extraction time (h)	B Enzymatic hydrolysis time (min)	C Solid-liquid ratio
−1	3	60	2
0	4	90	3
1	5	120	4

development tank saturated with the vapor of the development agent. When the solvent was expanded 10 to 14 cm on the thin plate, the thin plate was taken out, waiting for the developer to volatilize completely. Spray the thin plate evenly with 5% phosphomolybdate ethanol solution. After the ethanol volatilizes completely, heating the thin plate at 110°C for 8 to 10 min. Photographing was performed during 24 to 72 h of PCCL coloration (Zhou et al., 2020).

PC Content Analysis

High-performance liquid chromatography and evaporative light scattering detector (**HPLC-ELSD**) conditions were conducted with the approach adopted by (Kong et al., 2020) with minor modification. Quantitative analyses were performed with a Waters 2998 HPLC system. Separations were conducted using a 150 mm × 4.6 mm i.d., 5 μm, Intersil SIL-100A Column, and the injection volume was 20 μL. ELSD adopted split flow mode with air as atomizing gas and gas flow rate of 1.7 L/min. The mobile phase were that, A: acetonitrile-isopropanol (55: 5, V/V) and B: methanol-isopropanol (35: 5, v/v), with a flow rate of 0.8 mL/min, operating at 30°C. The linear gradient for solvent A was as follows: 0 to 25 min, from 70% change to 0%; 25 to 30 min, 0%; 30 to 32 min, from 0% change to 70%; followed by 8 min of column equilibration with 70% A. These conditions were based on (Kong et al., 2020). The solvents were filtered (0.22-μm pore size) into a vial before analysis.

Determination of Hydrophilic-Lipophilic Balance

Hydrophilic-lipophilic balance (**HLB**) described as a numerical value of the amphiphilic property of surfactants or emulsifiers (Park et al., 2018). HLB values of conjugates were determined by Griffin's method. Turpentine (HLB 6) and soybean oil (HLB 16) were mixed with different proportions to acquire oils with HLB values ranging from 6 to 16, and then PCCL were dissolved in mixed oil with different HLB values. After homogenization at 2,000 rpm for 15 min, the volume of emulsion in the centrifuge tube was measured, the average value of 3 parallel experiments was taken, and the emulsification was calculated according to the Equation (1). The HLB value of the mixture was decided as the HLB value of the oil that was found to be the most stable emulsion made by the assayed conjugate (Meng et al., 2021).

$$\text{Emulsification} = \frac{\text{volume of emulsion}}{\text{total volume of liquid}} \times 100\% \qquad (1)$$

Solubility of PC

The standard PC and n-hexane were accurately weighed to prepare PC-n-hexane solutions with the concentrations of 60, 100, 140, 180, and 220 mg/mL, respectively, for absorbance determination. The absorbance value of the supernatant was measured at 340 nm to calculate the concentration-absorbance linearity regression equation.

Excessive PCCL, soybean PC or egg PC was weighed into ethanol and glycerol respectively, placed into a 25° C water bath (Julabo SW23, Buch & Holm, Denmark) and shaken for 24 h until equilibrium was reached. And then, the samples were centrifuged at 5,000 r/min for 10 min to make the undissolved solid phase separated. The absorbance value of the supernatant was measured by absorbance at 340 nm, which was substituted into the regression equation to obtain the solubility of the sample.

Statistical Analysis

Excel 2016, origin 9.0 and design expert 8.0.6 were used to analyze and plot the data. SPSS 18.0 was used to analyze the data by single factor ANOVA graph base method. $P < 0.05$ indicated that there were significant differences between the two groups. Design expert 8.0.6 software was used for response surface analysis to obtain the optimal extraction value.

RESULTS AND DISCUSSION

Effect of Enzymatic Reaction Time

Figure 1A shows that when neutral proteinase and protamex proteinase were added to the enzymatic hydrolysis reaction, the extraction rate of PCCL increased first and then decreased sharply. The extraction rate of PCCL reached its highest at 60 min and 90 min, and the value was 59.2 and 46.63%, respectively. This was due to the specificity of the enzymes, which had different hydrolysis abilities to the substrate chicken liver protein. The protamex proteinase belongs to serine proteinase, which has the characteristics of both exonuclease and endonuclease. It follows the ordered reaction mechanism of 2 substrate reaction, and uses the exonuclease to hydrolyze the protein into peptides with smaller molecular weight. Then the hydrophobic amino acids are cut off from the end group of the polypeptide chain by exonuclease, and the polypeptide is cleaved into small fragments by endonuclease from the special peptide chain site in the middle of the polypeptide chain. Neutral proteinase has the highest activity in a neutral hydrolysis environment, and the reaction temperature was mild (Wang et al., 2021). It belongs to endonuclease, which has the advantages of mild conditions and short hydrolysis time. Therefore, it is a good proteolytic enzyme in terms of time cost. However, Neutral protease only hydrolyzes the peptide bond provided by hydrophobic macromolecular amino acids such as leucine, phenylalanine, and tyrosine. The protamex protease cannot only decompose hydrophobic groups, but also hydrolyze peptides from the N-end or C-end of peptide chain to amino acids. Thus, the enzymatic hydrolysis reaction of complex protease is more complete. After reaching the

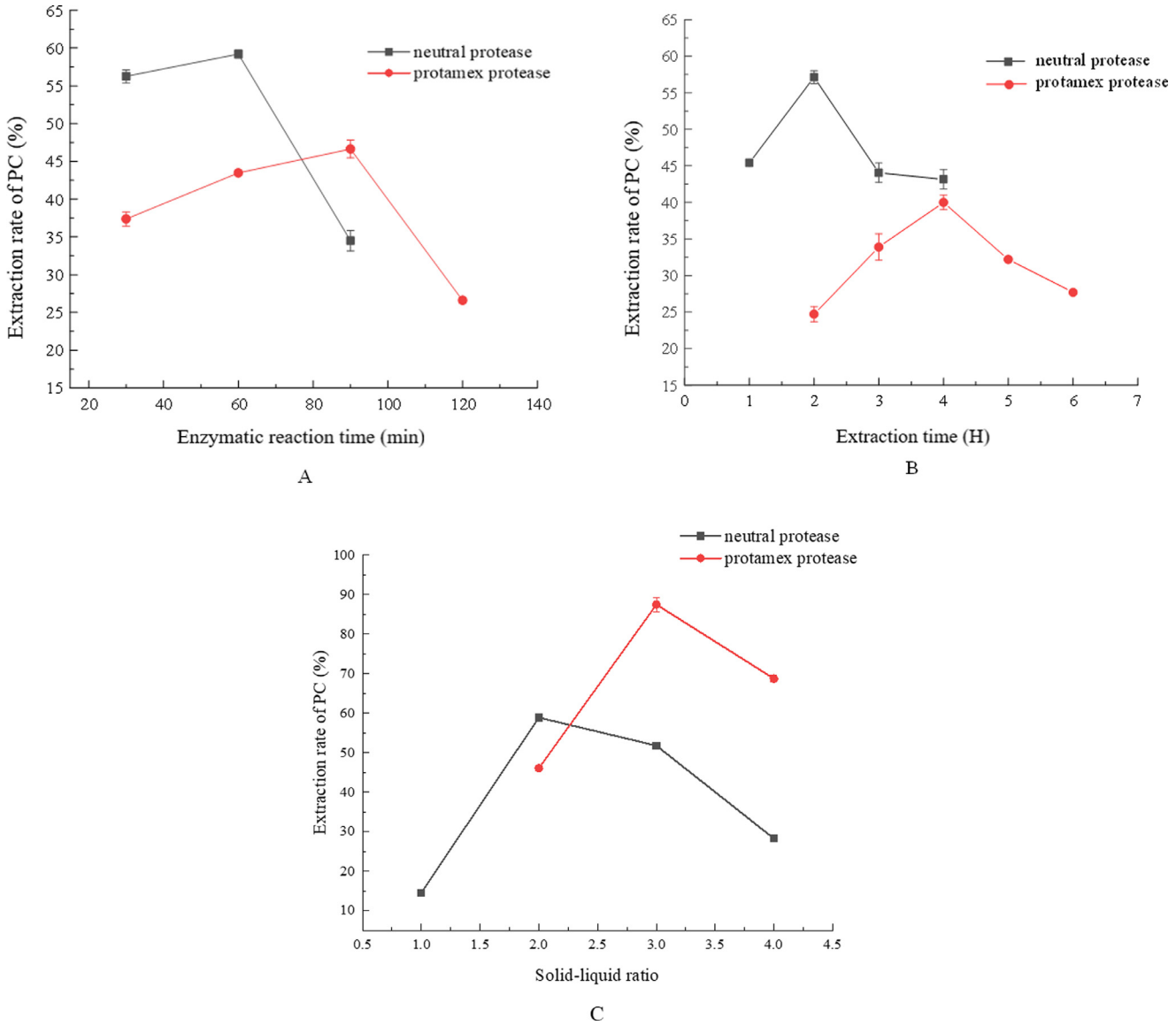

Figure 1. Extraction rate of PCCL: (A) effect of enzymatic reaction time; (B) effect of extraction time; (C) effect of material-liquid ratio. Abbreviation: PCCL, phosphatidylcholine from chicken liver.

highest value, the extraction rate decreased, which may be due to the cleavage of more and more macromolecular components and the transformation of insoluble components into soluble components with the extension of enzymatic hydrolysis time. On the one hand, it inhibited the enzymatic hydrolysis process; on the other hand, it hindered the contact between PC components and ethanol, which reduced the extraction rate.

Effect of Extraction Time

As shown in Figure 1B, when using neutral proteinase and protamex proteinase for enzymolysis, the extraction rate first increased and then decreased with the extention of time, reaching the maximum value of 57.1 and 39.3% at 2 h and 4 h, respectively. However, the extraction efficiency of neutral proteinase was significantly better than that of protamex proteinase ($P < 0.05$). Moreover, it was found in the process of the experiment that when the protamex proteinase was added to the enzymatic hydrolysis reaction, in the extraction reaction stage, 95% ethanol was added to the suspension after enzymatic hydrolysis, and the reaction was filtered in a water bath of 35°C, the extraction could not be completed at one time for 1 to 2 h, and the above steps need to be repeated for 2 times to make the reaction complete. This is because the protamex proteinase has more action sites than neutral protease, which makes the protein broken more completely, has a high degree of mixing with PC, reducing the filtration and separation efficiency. Therefore, in terms of time cost, neutral proteinase is a good choice.

Effect of Material-Liquid Ratio

As can be seen in Figure 1C, under the action of protamex proteinase and neutral proteinase, with the raise of solid-liquid ratio, the extraction rate of PCCL first

increased and then decreased. It was concluded that the highest extraction rate of PCCL was 87.5% when protamex proteinase was added and the ratio of solid to liquid was 1:3 (w/v). The ratio was lower than that reported by Wu and Wang (2004), they used 1:7 (w/v) solid to liquid to separate crude PC from soybean lecithin. This difference is possibly because PC content in eggs is higher than that in chicken liver, and more ethanol is needed to completely dissolve PC. When the amount of ethanol increases, the contact between solvent and solute is more sufficient, which improves the mass transfer rate, enhances the mass transfer ability, and is more conducive to the separation of PCCL from the oil binding. However, with the increasing amount of ethanol, the oil and other impurities in the raw material are also dissolved in the ethanol solution, resulting in the decrease of PCCL extraction rate. The results show that the extraction rate of PCCL does not increase with the increase of the solid-liquid ratio. If the amount of ethanol is insufficient, PCCL can't be completely separated out. On the contrary, if ethanol is added in excess, other components will be separated out and the extraction rate will decrease, which will bring difficulties to the subsequent concentration work. And excess ethanol will not only increase the cost, but also brings a waste of solvent and energy. Therefore, the material liquid ratio should be determined by considering the test result and cost.

Establishment of Mathematical Model and Significance Test

According to the results of single factor experiment, although the extraction efficiency of neutral proteinase is relatively high in a short period of time, the change of solid-liquid ratio can not be ignored. We selected the following parameters for RSM analysis: reaction time of 4 h, enzymatic hydrolysis time of 90 min, 1: 3 (w/v) of solid-liquid ratio, using protamex proteinase. The quadratic multinomial regression equation with extraction rate as the objective function was obtained as follows:

$$Y = 87.63 - 6.79A - 6.06B + 8.44C - 3.30AB - 2.63AC + 2.68BC - 13.44A^2 - 15.17\ B^2 - 28.71\ C^2 \quad (2)$$

In the equation, Y is the extraction rate of PCCL, A, B, and C represent the extraction reaction time, enzymatic hydrolysis reaction time, and solid-liquid ratio, respectively. The absolute value of each coefficient in the equation reflects the influence degree of each factor on the value of the objective function. As seen in Table 3, the results of significance test of regression equation coefficient of the model showed that the first term a ($P < 0.001$), B ($P < 0.001$), and C ($P < 0.001$) were highly significant; the interaction terms AB ($P = 0.0171$), AC ($P = 0.0427$), and BC ($P = 0.0398$) were significantly; The quadratic terms A^2 ($P < 0.001$), B^2 ($P < 0.001$), and C^2 ($P < 0.001$) were highly significant (Table 2), which indicated that the extraction reaction time, enzymolysis reaction time, solid-liquid ratio, extraction reaction time and enzymatic hydrolysis reaction time, extraction reaction time and solid-liquid ratio, enzymatic hydrolysis reaction time, and solid-liquid ratio had significant effects on the extraction rate of PCCL.

The response surface plots show the interactive effects between extraction reaction time and enzymatic hydrolysis reaction time, extraction reaction time and solid-liquid ratio, extraction reaction time, and solid-liquid ratio. As shown in Figure 2A, it can be seen from the steepness of the response surface and the shape of the contour. When the extraction time and enzymatic hydrolysis time reached about 4 h and 90 min, respectively, the extraction rate of PCCL increased with the extension of time and the further increase of these two parameters led to the decrease of extraction rate. Similarly, the interaction between extraction time and solid-liquid ratio, enzymatic hydrolysis time and solid-liquid ratio were positively correlated at first and then negatively correlated with the increase of experimental parameters (Figures 2B and 2C). The results showed that the three interactions had effects on the extraction rate of PCCL, and the maximum value was found in the experimental range. Specifically, in the second-stage optimization, the optimum conditions of extracting PCCL were determined by calculation as follows: reaction time 3.75 h, enzymatic hydrolysis time 85.22 min, 1: 3.15 of solid-liquid ratio. Under these conditions, the predicted yield of PCCL was 89.59%, it was analogue to the reported study (Xie et al., 2017; Liu et al., 2018).

Verification of Extraction Conditions

Three parallel experiments were carried out to verify the repeatability and accuracy of the experiment. The average value of the three experiments was 88.92%, and the relative error was 0.747%, which was in good agreement with the predicted value. It showed that this condition had high practical application value and could be used for the extraction process of PCCL.

Table 2. ANOVA of regression coefficients.

Source	Sum of Squares	df	Mean Square	F Value	*P*-value Prob>F	
Model	7,046.13	9	782.90	173.66	<0.0001	significant
A	368.56	1	368.56	81.75	<0.0001	
B	294.03	1	294.03	65.22	<0.0001	
C	569.87	1	569.87	126.40	<0.0001	
AB	43.56	1	43.56	9.66	0.0171	
AC	27.56	1	27.56	6.11	0.0427	
BC	28.62	1	28.62	6.35	0.0398	
A^2	760.45	1	760.45	168.68	<0.0001	
B^2	968.84	1	968.84	214.90	<0.0001	
C^2	3,471.55	1	3,471.55	770.04	<0.0001	
Residual	31.56	7	4.51			
Lack of fit	26.16	3	8.72	6.47	0.0515	not significant
Pure error	5.39	4	1.35			
Cor total	7,077.69	16				

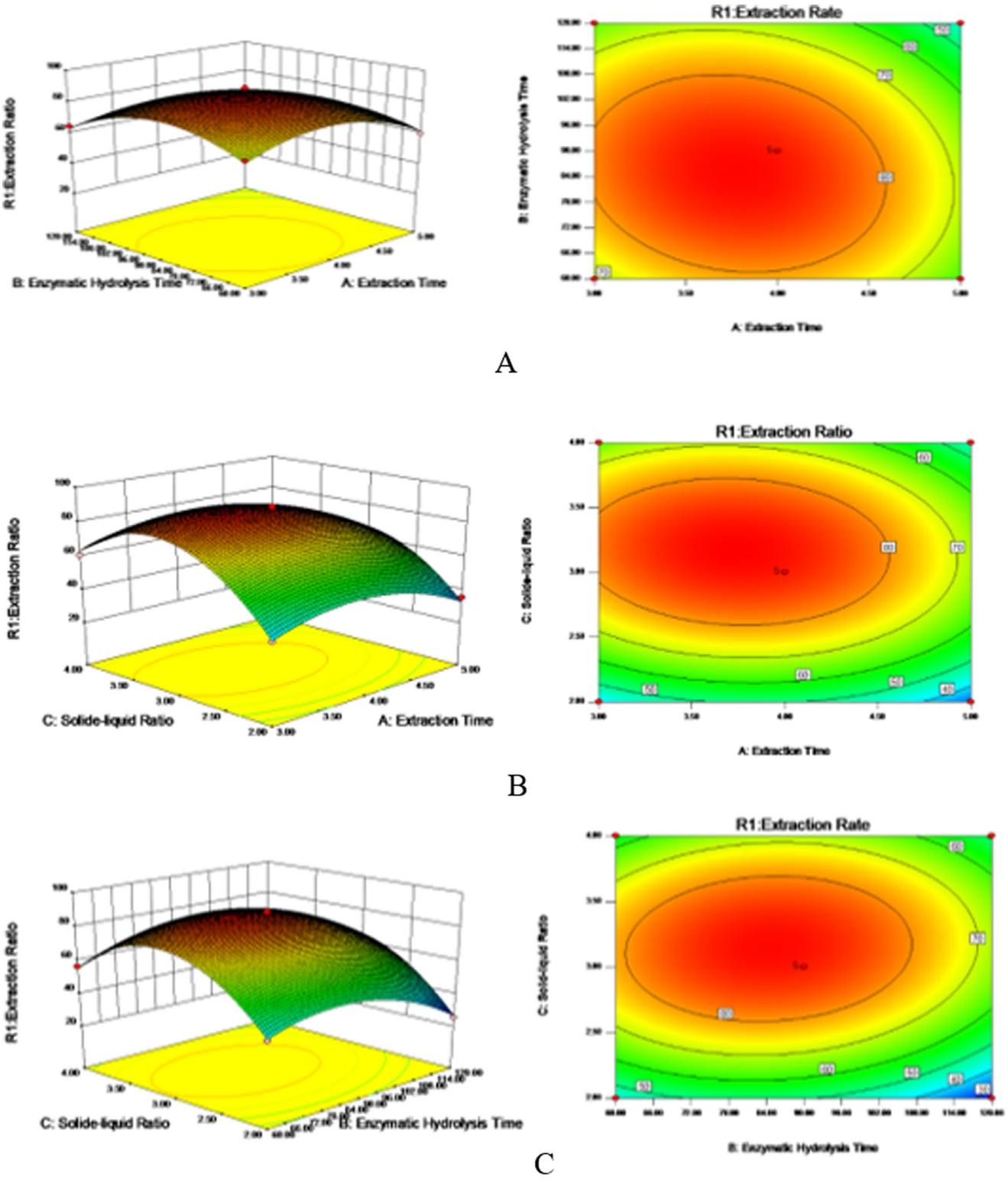

Figure 2. Response surface plots of extraction rate: (A) interaction between extraction reaction time and enzymatic hydrolysis time; (B) interaction between extraction reaction time and solid-liquid ratio; (C) interaction between enzymatic hydrolysis time and solid-liquid ratio.

Qualitative Analysis of PCCL

The TLC method depended on the difference between values of retardation factor (**Rf**) of PC, PE, PI, and other substances because of the difference in their migration rates and in their polarities on the polar silica plates (Sobstyl et al., 2020). As shown in Figure 3, from left to right are PC standard and sample PCCL, through the measurement of PC standard, the RF value of PCs is 0.78. The PCCL showed the same color spots on the corresponding position with the standard sample, indicating that the main component of phospholipid extracted

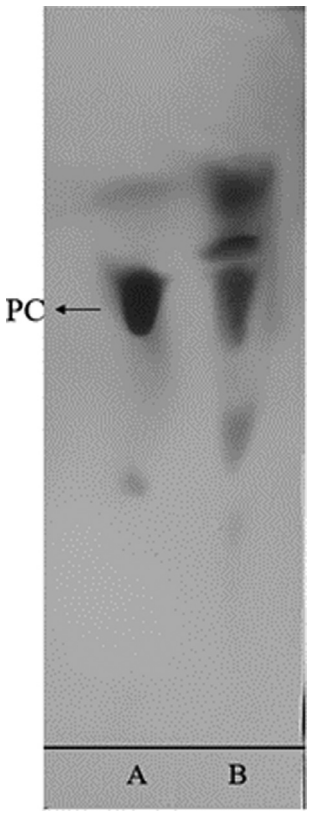

Figure 3. The TLC chromatogram: (A) PC standard; (B) PCCL sample. Abbreviations: PC, phosphatidylcholine; PCCL, phosphatidylcholine from chicken liver; TLC, Thin layer chromatography

from chicken liver was PC. Except that, during the development process, the solvent with low boiling point and weak polarity volatilizes on both sides of the thin-layer plate, which makes the proportion of the developing agent on the thin-layer plate inconsistent and the polarity changes, resulting in edge effect. Nevertheless, the sensitivity of the present method could tally with the destination for the qualitative analysis of the PCCL. In addition, TLC is a stable, environment friendly and cost-efficient chromatographic separation technique, which coincides with the requirements of green energy saving in this study.

HPLC Analysis

Based on the polarity of the head group and the hydrophobicity of fatty acid acyl chain, the separation and quantification of PCCL were carried out by HPLC. According to Zhou's method, PC standard solution was prepared and eluted by methanol- acetonitrile. The results reflected that adding a little amount of isopropanol in the mobile phase could extend the distance between the solvent peak and the target peak to improve the peak shape, therefore, methanol-isopropanol, and acetonitrile-isopropanol gradient elution was selected (Ferreyra et al., 2021). To quantify PCCL production, a calibration curve of PC production concentration versus

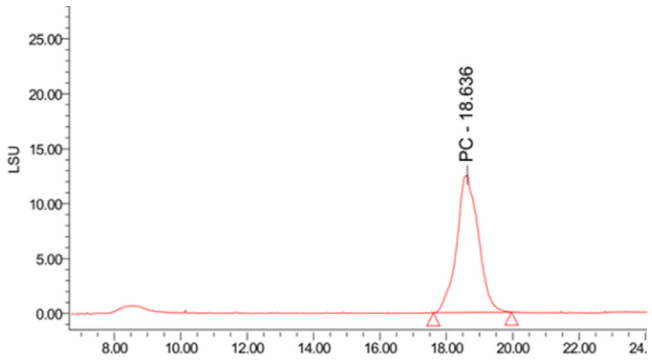

Figure 4. HPLC chromatogram of PCCL (wavelength: 210 nm). Abbreviations: HPLC, High-performance liquid chromatography; PCCL, phosphatidylcholine from chicken liver.

peak height was plotted using the highly purified PC (≥98.0%) as a standard. The peaks at 8.5 min and 18.6 min in the HPLC-ELSD chromatogram were continually detected from the early stage, which were identified as PE and PC, respectively. The peak shape of PC was good under the optimized mobile phase system, which was suitable for quantitative analysis. As shown in Figure 4, the retention time of the peak at 18.6 min was identical to the standard PC, the peak area of HPLC chromatogram is 17.6 to 19.9 min. The concentration of PCCL was calculated as 0.89 mg/mL, which have the similar purity with the reported (Wu and Wang, 2004; Patil et al., 2010; Subra-Paternault et al., 2015).

HLB of Different PCs

The HLB value is one of the most widely used index of surfactants, which was defined by Griffin as the ratio of a surfactant's hydrophilicity to its hydrophobicity (Wu et al., 2021). The HLB value of non-ionic surfactants is generally between 0 and 20, and high HLB value indicates strong hydrophilicity and vice versa (Gad and Khairou, 2008). PC are amphiphilic because they have a hydrophilic head and hydrophobic fatty acid tail (Price et al., 2018), which makes PC-enriched component have the advantage of improved oil-in-water (o/w) emulsifying properties that are needed by food, pharmaceutical, and cosmetic industries (Verkempinck et al., 2018; Xie and Dunford, 2019). Experiments show that, the proportions of emulsion layer are 0.455, 0.801, 0.267, respectively, and their HLB values were determined and found to be from 10 to 13. Among them, the HLB value of PCCL was the lowest, and that of soybean PC was the highest, which were 10 and 13. It showed that all 3 kinds of PCs were suitable to be used as oil-in-water (o/w) emulsifier, and the PCCL has good lipophilicity, which shows the better demulsification and defoaming properties (Wang et al., 2018), it is more suitable as a natural surfactant of oil soluble substances. The experimental results have implications in the selection of PC according to different needs (Shen et al., 2020).

Table 3. Solubility of PC in ethanol and glycerol.

	Soybean PC		Egg yolk PC		PCCL	
Dissolvant	Ethanol	Glycerol	Ethanol	Glycerol	Ethanol	Glycerol
Dilution ratio	0	10	0	10	10	0
Absorbance	0.8435	1.0950	0.6585	1.5087	0.5190	0.8075
Solubility (g/mL)	0.1012	1.3433	0.0769	1.8872	0.5850	0.0965

Abbreviations: PC, phosphatidylcholine; PCCL, phosphatidylcholine from chicken liver.

Solubility of PC in Ethanol and Glycerol

PC has the functions of moisturizing and antioxidation. The addition of PC in cosmetics can protect skin's regeneration vitality and increase its skin luster. On the other hand, the above research showed that PCCL had good hydrophilicity and lipophilicity. It is often used in making cosmetics such as cream, eye cream and lipstick, consequently (Jatoi et al., 2017). The solubilities of several PCs when exposed to common cosmetic raw materials (ethanol and glycerol) have been studied. Substituting into the regression equation $Y = 0.0076X + 0.0744$, showing that in ethanol, the solubility of PCCL was the highest (0.5850 g/mL), followed by soybean PC (0.1012 g/mL), and egg PC is the lowest (0.0769 g/mL). Therefore, an appropriate amount of PCCL can be added as an antioxidant in alcoholic beverages and cosmetics. When glycerol was used as solvent, the solubility of soybean PC and egg PC increased significantly, the values are 1.3433 g/mL and 1.8872 g/mL, respectively. But the solubility of PCCL was only 0.0965 g/mL (Table 3). In summary, the appropriate solvent should be selected to dissolve PCs according to different purposes.

CONCLUSIONS

In this work, ultrasound-assisted enzymatic extract was used in the extraction of PC from chicken liver. This research showed the optimum conditions of extracting PC from chicken liver were under the action of protamex proteinase, reaction time of 3.75 h, enzymatic hydrolysis time of 85.22 min and 1: 3.15 of solid-liquid ratio. Under the optimum conditions, the yield and concentration of PC was 88.92% and 0.89 mg/mL, respectively. The application prospect of the PC extraction from chicken liver was discussed. By analyzing the solubility and emulsifying properties of PCs from different sources, we found that, when ethanol is used as solvent, PCCL has better solubility. Hence, the potential of PCCL as a nontoxic emollient and antioxidant adding to cosmetics was considered. Otherwise, that all three kinds of PCs were suitable to be used as oil-in-water (o/w) emulsifier, among them, PCCL has the most lipophilic property. It can be used as a natural defoamer in the production of bean products, wine, soy sauce and other foods. This research could establish PCCL viability as an alternative to currently available extraction material of PC. It provides a new idea for searching an environmentally friendly method to reuse by-products and develop animal PC.

ACKNOWLEDGMENTS

This study was supported by the National Natural Science Foundation of China (31901612), and the China Agriculture Research System (CARS-41).

DISCLOSURES

The authors declare that they have no conflict of interest in this work.

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