Eur Food Res Technol (2013) 237:811–817
DOI 10.1007/s00217-013-2057-z

ORIGINAL PAPER

Optimization of magnetic immobilized phospholipase A_1 degumming process for soybean oil using response surface methodology

Dianyu Yu · Ying Ma · Lianzhou Jiang · Walid Elfalleh · Min Shi · Lizhi Hu

Received: 18 April 2013 / Revised: 30 June 2013 / Accepted: 1 July 2013 / Published online: 17 July 2013

Abstract This study focused on optimization of processing conditions of enzymatic degumming process for soybean oil using phospholipase A_1 immobilized onto magnetic nanoparticles. A response surface methodology was developed and used to obtain optimum processing conditions. Four variables (temperature, reaction time, enzyme dosage, and added water) were investigated based on two response functions (phosphorus and free fatty acids (FFA) contents in degummed soy oil). For each response, second-order polynomial models were developed using multiple linear regression analysis. The optimum operating parameters of enzymatic degumming process were as follows: temperature of 56 °C, reaction time of 6.3 h, enzyme dosage of 0.10 g/kg, and added water of 2.13 ml/100 g. According to these optimum conditions, final residual phosphorus and FFA contents of degummed soy oil were reduced, respectively, to 10.38 mg/kg and 1.09/100 g using magnetic immobilized phospholipase A_1. This finding is applicable for the physical refining of soybean oil or refining crude oils from field- and frost-damaged beans which have high content of non-hydratable phosphatides.

D. Yu · Y. Ma · W. Elfalleh (✉)
School of Food Science and Engineering, Harbin Institute of Technology, Harbin 150090, China
e-mail: walid.elfalleh@fst.rnu.tn

D. Yu · L. Jiang · M. Shi
School of Food Science, Northeast Agricultural University, Harbin 150030, China

L. Hu
National Soybean Engineering Technology Research Center, Northeast Agricultural University, 201 Gongbin Road, Xiangfang District, Harbin 150030, Heilongjiang, China
e-mail: hlz035d832n@yeah.net

Keywords Soybean oil · Enzymatic degumming · Optimization · Magnetic immobilized phospholipase A_1 · Response surface methodology

Introduction

The complete edible oil refining process comprises a series of discrete processing steps, such as degumming, neutralization, bleaching and deodorization, and so on. The general objective of refining is to remove undesirable components (phosphatides, gums, free fatty acids, colors, undesirable flavors and odors, trace metals and oxidation products, etc.) that have an adverse effect on the overall oil quality. Degumming is the first step in the refining process of vegetable oils, which mainly removes phospholipids and mucilaginous gums [1]. The presence of substantial amounts of phospholipids can cause oil discoloration and serve as precursor of off-flavors. Therefore, the removal of nearly all of the phospholipids is essential for the production of high-quality finished oil [2]. Phosphatides can be present in crude oils in the free hydratable (HP) or in the non-hydratable form (NHP). Phosphatidylcholine (PC) exists in oils as a hydratable zwitterion, while phosphatidylinositol (PI) exists as a complex with potassitol and magnesium, which is fully hydratable due to the hydrophilic inositol group. The non-hydratable phosphatides are present mainly as calcium and/or magnesium (Ca/Mg) salts of phosphatidic acid (PA) and PE. The non-hydratable phosphatides are more difficult to remove and have to be transformed by treatment with a concentrated acid. In industrial practice, edible oils may be refined by a series of processes that can be grouped together as "chemical refining" or "physical refining." The former involves degumming, chemical neutralization, bleaching, and deodorization, while the latter requires only bleaching

and steam distillation (deodorization). The physical refining process can offer important advantages to the refiner, such as higher oil yield, reduction of the use of chemicals (phosphoric acid, sulfuric acid, caustic soda, etc.), and reduction in water use and effluent of 75 and 95 %, respectively. This may reduce effectively the environmental impact [3]. Degumming is especially critical in the case of physical refining, which requires almost 100 % removal of the phosphatides [4].

In addition, since unfavorable weather conditions, soybeans are not harvested promptly after maturity but are left exposed to rain or damp weather frequently in Heilongjiang Province of China. The field- and weather damages result in very high moisture content of the beans and a lot of green beans or immature-harvested beans. As a result of severe frost-damaged beans, refining crude oils from field- and frost-damaged beans is more costly and more difficult than normal because of high content of non-hydratable phosphatides. In very severe cases, the soybean oil from frost- and field-damaged beans would not degum properly by normal degumming method or adding chemicals and finally yielded poor quality products.

Enzymatic degumming is suitable for physical refining process, because the degumming is especially critical in the case of physical refining which requires very low phosphorus (preferably <10 ppm) and iron (preferably <0.2 ppm) contents in the pretreated oil [5]. Therefore, the enzymatic degumming process is necessary to be used for physical refining process or refining crude oils from field- and frost-damaged beans which have high content of non-hydratable phosphatides.

Enzymatic degumming was firstly reported in 1990s by Roehm and Lurgi concerning the commercial "EnzyMax@ process" [6, 7], in which the phospholipase A_1 or A_2 was used to convert non-hydratable phospholipides into their hydratable forms (lysophospholipids). This process consists of three important steps: adjusting the pH of the oil with buffer, the addition of the enzyme solution and carrying out the enzyme reaction, and separating gum/sludge from the oil. Enzymatic degumming process has many advantages than traditional degumming, i.e., in addition to the reduction in the amounts of acid and base used, and a reduction in wastewater generated during the refining process, an enhancement in product yields and a reduction in operating costs can be achieved [8]. Therefore, enzymatic degumming process offers a safe biological route and eco-friendly solution for the industrial process, since it can successfully reduce phosphorus levels to less than 10 mg/kg on the degumming process of vegetable oils [9–11].

Because immobilized enzymes are more efficient than free enzymes in industrial practice, many techniques have been used for enzyme immobilization, such as attachment, embedding, and encapsulation. The nanoparticle can provide a larger surface area for the attachment of enzymes, leading to higher enzyme loading per unit mass of particles [12]. Magnetic nanoparticles are a new type of functional polymer materials developed recently, and they are easy and rapid to separate the enzyme from the reaction medium in a magnetic field, increasing loading amount of the biomolecules immobilized on magnetic particles and improving the stability of immobilized biomolecules [13–15].

The response surface methodology (RSM) has been demonstrated to be a powerful tool for determining the factors and their interactions, which allow process optimization to be conducted effectively [16]. This method is the preferred experimental design for fitting polynomial model and to analyze the response surface of multifactor combinations. Furthermore, it is fast and more economical method for gathering research results than classic, one variable at a time or full-factors experimentation.

In this study, the magnetic immobilized phospholipase A_1 and a response surface methodology were used to evaluate the effects of four factors on enzymatic degumming process of soybean oil. These factors are temperature, reaction time, enzyme dosage, and added water. The two response functions were the residual phosphorus and FFA contents of soybean oil. The main goal is to optimize the conditions of enzymatic degumming process and to obtain the minimum phosphorus and FFA contents of enzymatic degumming oil.

Materials and methods

Materials

Water-degummed soybean oil with a phosphorus content of 138 mg/kg and FFA content of 0.93/100 g as oleic acid was supplied by the Fukang Oil and Fat Co. (Harbin, China). Phospholipase A_1 (Lecitase@ Ultra EC 3.1.1.3) was obtained from Novozymes A/S (Bagsvaerd, Denmark), with the activity of 6,800 U/g. All other chemicals were of analytical grade.

Immobilization of phospholipase A_1

A 1 g magnetic nanoparticle was immersed in sodium phosphate buffer (0.01 M, pH 7.0) for 24 h, and then, the magnetic nanoparticles were separated from the buffer. After the mixture of 50 ml of sodium phosphate buffer (0.01 M, pH 7.0) was mixed with 5-ml-diluted phospholipase A_1 solution at a speed of 180 rpm, the magnetic nanoparticles were separated. Next, the magnetic immobilized phospholipase A_1 was washed by the buffer, and the obtained magnetic immobilized phospholipase A_1 was dried in a vacuum desiccator at 30 °C for 4 h and was storied at 4 °C before use.

The efficiency of magnetic immobilized phospholipase A_1 has been reported previously by Yu et al. [17]. The immobilized phospholipase A_1 process retained 2,066.67 U/g activity with 64.7 % immobilization efficiency.

The operational stability of magnetic immobilized phospholipase A_1 was tested by recycling in water degumming oil. The activity of the first cycle was defined as 100 %. The activity of the immobilized phospholipase A_1 decreased continuously with increasing number of cycles. In a previous investigation of phospholipase A_1 recycling, results showed that after 10 cycles through the soybean oil degumming process at 55 °C, the immobilized phospholipase A_1 still possessed more than 80 % of its initial activity [17] .

Enzymatic degumming process

A 300 ml of soybean oil and 0.13 ml of citric acid solution (45 %) were placed into reactor, and the oil was heated at 80 °C and stirred at 900 rpm for 5 min. Then, the oil was cooled to 60 °C and added to NaOH solution (4 %) adjusting the pH approximately 5.0 in the water/sludge phase while stirring at 900 rpm. Afterward, about 3 ml of water was added to the oil and stirred at 30 rpm for 20 min. After that, the magnetic immobilized phospholipase A_1 solution was added to the oil, and then, the mixture was maintained at 60 °C and stirred at 30 rpm for 10 h. The samples for phosphorus and FFA analysis were drawn at 1 h intervals, and the reaction mixture was centrifuged (approximately 6,000 g) to separate the magnetic immobilized phospholipase A_1, mucilaginous gums, and degummed soy oil.

Analysis of phosphorus and FFA contents

The residual phosphorus content of the oil samples was determined according to AOCS method ca 12–55 [18]. FFA content of the degummed oil was assayed by AOCS ca 5a-40 [19]. All experiments were carried out in triplicate.

pH determination

About 2 ml of water in oil emulsion was mixed with distilled water of 2 ml. After phase separation, top layer oil was pipetted off. The pH in aqueous phase was measured with pH meter electrode. Measurements were transformed to corrected pH values by formula ($pH_{corrected} = pH_{measured} - 0.26$), to compensate for the dilution effect.

Experimental design and statistical analysis

Response surface methodology was performed to optimize the process of enzymatic oil degumming. The four selected factors were temperature (X_1), reaction time (X_2), enzyme dosage (X_3), and added water (X_4). The phosphorus and FFA contents were taken as the responses. A set of 32 experiments with four variables were required. For statistical calculations, the relation between the coded values and actual values is described by Eq. (1):

$$\chi_i = \frac{X_i - X_0}{\Delta X} \quad (1)$$

where χ_i is the coded value of the independent variable, X_i is the actual value of the variable, X_0 is the actual value at the center point, and ΔX is the step change in the variable X_i. The design matrix is shown in Table 1. The phosphorus

Table 1 Box-Behnken experimental design in coded and actual levels of variations

Run.	X_1	X_2	X_3	X_4	P (mg/kg)	FFA (g/100 g)
1	−1	−1	0	0	15.28	1.006
2	1	−1	0	0	12.35	1.048
3	−1	1	0	0	13.18	1.0478
4	1	1	0	0	14.62	1.0785
5	0	0	−1	−1	17.73	0.992
6	0	0	1	−1	12.93	1.055
7	0	0	−1	1	17.80	1.015
8	0	0	1	1	11.05	1.080
9	−1	0	0	−1	15.18	1.020
10	1	0	0	−1	12.21	1.055
11	−1	0	0	1	11.00	1.045
12	1	0	0	1	11.65	1.082
13	0	−1	−1	0	18.65	0.986
14	0	1	−1	0	16.73	1.015
15	0	−1	1	0	11.95	1.048
16	0	1	1	0	10.93	1.878
17	−1	0	−1	0	16.13	0.980
18	1	0	−1	0	17.90	1.015
19	−1	0	1	0	13.25	1.0455
20	1	0	1	0	11.40	1.080
21	0	−1	0	−1	14.80	1.023
22	0	1	0	−1	12.85	1.055
23	0	−1	0	1	12.2	1.048
24	0	1	0	1	11.18	1.079
25	0	0	0	0	10.60	1.075
26	0	0	0	0	10.43	1.074
27	0	0	0	0	12.57	1.078
28	0	0	0	0	11.23	1.076
29	0	0	0	0	10.89	1.075
30	0	0	0	0	11.90	1.073
31	0	0	0	0	12.00	1.081
32	0	0	0	0	11.34	1.072

X_1, temperature (°C); X_2, reaction time (h); X_3, enzyme dosage (g/kg); X_4, added water (ml/100 g)

−1, 1, and 0 represent the encoding of the data values corresponding to each optimized single factor, respectively

and FFA contents are taken as the responses. The quadratic equation for the variables is shown in Eq. (2):

$$Y = \beta_0 + \sum \beta_i \chi_i + \sum \beta_{ii} \chi_i^2 + \sum \beta_{ij} \chi_i \chi_j \quad (2)$$

where Y = predicted response. β_0 represents interception. β_i, β_{ii}, and β_{ij} are the linear, quadratic, and interaction coefficients, respectively. χ_i and χ_j are the independent variables.

Equation (2) was used to build surfaces for variables. A software Box-Behnken 7.1.3 (USA) was used to obtain the coefficients of the quadratic polynomial model. The quality of the fitted model was expressed by the coefficient of determination R^2, and its statistical significance was checked by F test. By keeping two variables at their central levels, 3D and contour plots of two factors against the responses (phosphorus or FFA contents) of oil were drawn.

Result and discussion

Prior to RSM design, the single factor studies have been conducted and obtained suitable ranges of each independent variable. The results indicate that optimum ranges were as follows: the temperature (X_1) from 50 to 60 °C; reaction time (X_2) from 5 to 7 h; enzyme dosage (X_3) from 0.08 to 0.12 %; and added water (X_4) from 1.5 to 2.5 %.

Fitting models and contour plots

The effects of temperature (X_1), reaction time (X_2), enzyme dosage (X_3), and added water (X_4) on the phosphorus and FFA contents for enzymatic degumming process were investigated with RSM. The observed values for the phosphorus and FFA contents at different combinations of the independent variables are presented in Table 1.

An analysis of variance (ANOVA) was conducted to determine the significant effects of process variables on each response. Table 2 presents the ANOVA table for two response functions. The coefficient of determination (R^2) of the model for phosphorus content was 0.9374 and Adj-R^2 was 0.8858 which indicated that the model adequately represented the real relationship between the chosen parameters. The coefficient of determination (R^2) of the model for FFA content also indicated better results than the model for phosphorus contents, i.e., R^2 was 0.9967 and Adj-R^2 was 0.9940. Furthermore, the results of the error analysis for the both phosphorus and FFA contents indicated that the lack of fit was insignificant ($P < 0.05$). The coefficient variation (C.V.) for both phosphorus and FFA contents was less than 7 % which indicated the reproducibility of the model.

The regression coefficients of the intercept, linear, quadratic, and interaction terms of the model were calculated using the least square technique and are given in Table 3. It was evident that all the independent variables considered exhibited significant effects ($P > 0.05$). There were significant interaction ($P < 0.05$) between temperature, reaction time, enzyme dosage, and added water for the phosphorus and FFA contents, respectively.

Optimization of enzymatic degumming parameters

Phosphorus content

The phosphorus content of water-degummed soybean oil (138 mg/kg) was reduced to 10.38 mg/kg after enzymatic

Table 2 Results of analysis of variance for phosphorus and FFA contents

Source	SS	DF	MS	F value	P value
P					
Model	175.02	14	12.50	18.18	<0.0001
Residual	11.69	17	0.69	–	–
Lack of fit	7.85	10	0.78	1.43	0.3269
Pure error	3.85	7	0.55	–	–
Total	186.71	31	–	–	–
R^2	0.9374		Adj-R^2	0.8858	
C.V. %	6.26		PRESS	50.21	
FFA					
Model	0.031	14	2.197×10^{-3}	367.25	<0.0001
Residual	1.017×10^{-4}	17	5.981×10^{-6}	–	–
Lack of fit	4.019×10^{-5}	10	4.019×10^{-6}	0.46	0.8734
Pure error	6.150×10^{-5}	7	8.786×10^{-6}	–	–
Total	0.031	31	–	–	–
R^2	0.9967		Adj-R^2	0.9940	
C.V. %	0.23		PRESS	3.118×10^{-4}	

SS sum of squares, *DF* degree of freedom, *MS* mean squares

Table 3 Regression coefficients of the second-order polynomial model for the responses variables

Coefficients	*P* (mg/kg)	FFA (g/100 g)
β_0	324.179	−2.83325
Linear		
β_1	−5.42250	0.077213***
β_2	−26.93583*	0.19762***
β_3	−860.70833***	15.23833***
β_4	−27.86167*	0.17487 ***
Quadratic		
β_{11}	0.038433*	-6.41167×10^{-4}***
β_{22}	1.03208*	−0.013554***
β_{33}	6,245.83333***	−72.32292***
β_{44}	2.05833	−0.040917***
Interaction		
β_{12}	0.21850*	-5.65000×10^{-4}*
β_{13}	−9.05000*	-1.25000×10^{-3}
β_{14}	0.36200*	2.00000×10^{-4}
β_{23}	11.25000	0.13875*
β_{24}	0.465000	-4.00000×10^{-4}
β_{34}	−48.75000	0.050000

Analysis has been performed using coded units

* *P* value <0.05, *** *P* value <0.01

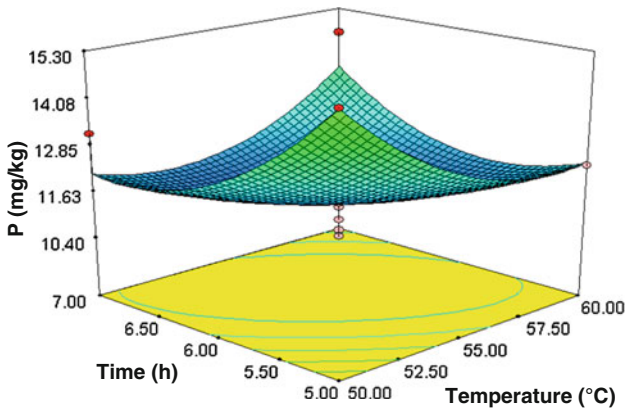

Fig. 1 Response surface and contour plots for the effect of temperature (X_1) and reaction time (X_2) on the residual phosphorus content, enzyme dosage (X_3), and added water (X_4) kept in central points

degumming. The ANOVA table (Table 3) for phosphorus content shows a high Adj-R^2 which indicates the suitability of second-order polynomial to predict. Among the four variables, reaction time had the highest effect on phosphorus content. As presented in Fig. 1, the phosphorus content decreased with increasing reaction time. The residual phosphorus content of the degummed oil decreased to less than 10.40 mg/kg when the reaction time was increased to 6.0 h. These results are in close agreement with our earlier results of immobilized phospholipase A_1 in

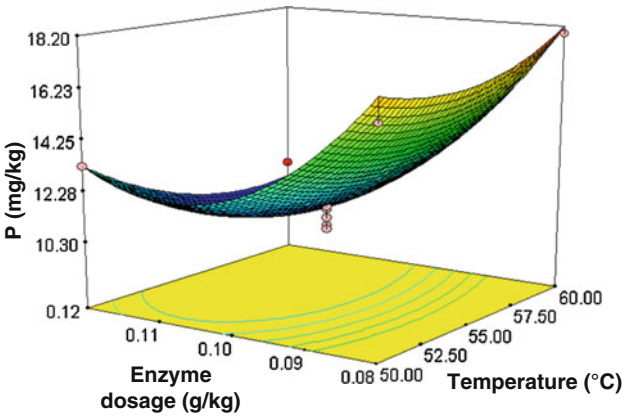

Fig. 2 Response surface and contour plots for the effect of temperature (X_1) and enzyme dosage (X_3) on the residual phosphorus content, added water (X_4), and reaction time (X_2) kept in central points

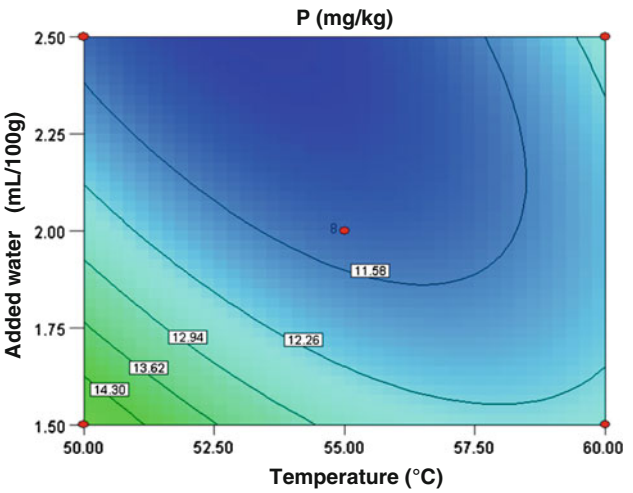

Fig. 3 Response surface and contour plots for the effect of added water (X_4) and temperature (X_1) on the residual phosphorus content, reaction time (X_2), and enzyme dosage (X_3) kept in central points

which Yu et al. [20] described that the phosphorus content was reduced to less than 10 mg/kg over 6 h using immobilized phospholipase A_1–CAC. Figure 2 indicates that the residual phosphorus content decreased when enzyme dosage increases.

Figure 3 shows the significant quadratic effect of temperature on residual phosphorus content. In the range of temperature from 50 to 56 °C, the content of residual phosphorus decreased with temperature increasing, probably due to the increased hydrolytic activity of the enzyme. The content of residual phosphorus increased with the further increasing of temperature, which could be due to partial denaturation of the enzyme and loss of its hydrolytic activity.

From the results, the phospholipase A_1 immobilized on magnetic nanoparticles improved the thermal stability of

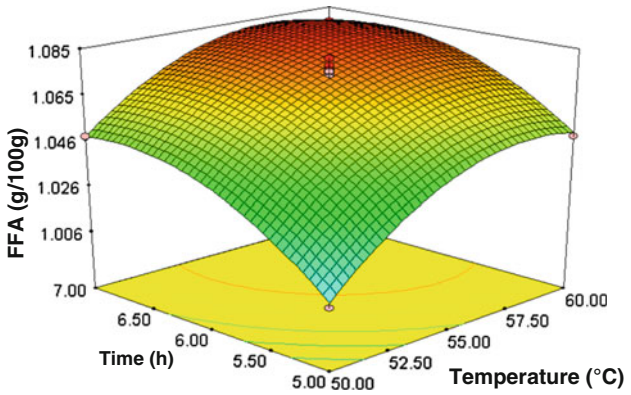

Fig. 4 Response surface and contour plots for the effect of reaction time (X_2) and temperature (X_1) on the FFA content, added water (X_4), and enzyme dosage (X_3) kept in central points

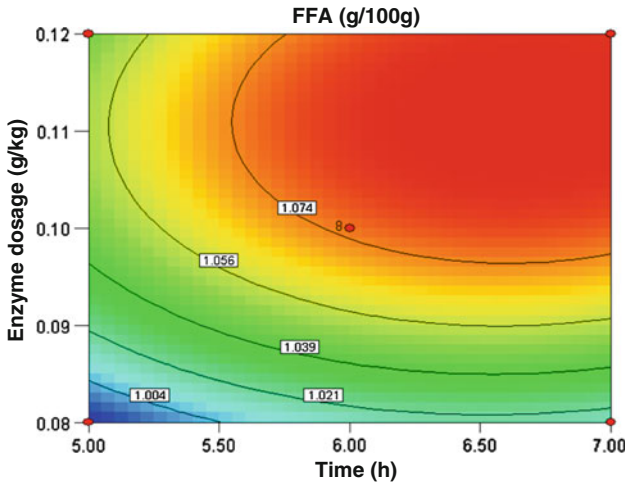

Fig. 5 Response surface and contour plots for the effect of reaction time (X_2) and enzyme dosage (X_3) on the FFA content, temperature (X_1), and added water (X_4) kept in central points

immobilized enzyme. Bornscheuer [21] reported that the shift in optimal temperature toward higher values could be due to the immobilization of the enzyme. Immobilization improved thermal stability and resulted in formation of the enzyme substrate complex which hindered the access of substrates to the active site. Figure 3 also illustrates the negative effect of added water on residual phosphorus content in the oil. This can be attributed to hydrolase nature of the used enzyme and direct contribution of water in the hydrolysis of phospholipids.

FFA content

Figure 4 indicates that the reaction time had the significant effect on FFA content. FFA content increased with increasing reaction time: The increase of FFA content from 0.93 to 1.09/100 g was due to the fatty acids released during enzymatic hydrolysis of the phospholipids present

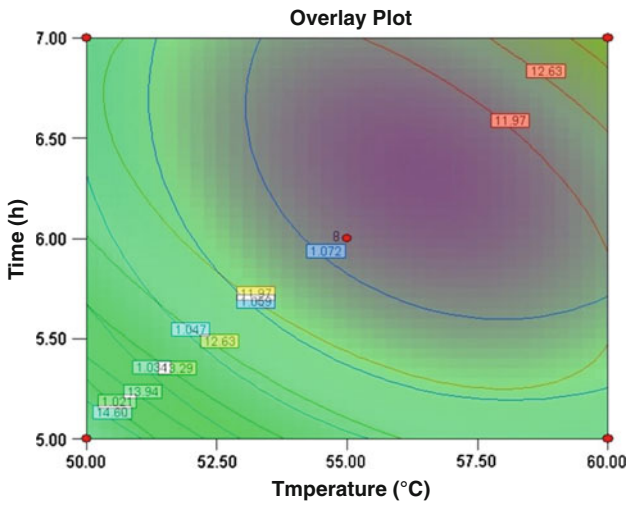

Fig. 6 Response surface and contour plots for optimum region identified by overlaying plots of the two responses (P and FFA) as functions of reaction time and temperature

in the oil. Yang et al. [9] reported that enzymatic degumming process caused slight increase of FFA content in the rapeseed and soybean oils. Reaction temperature shows significant effect on FFA content: FFA content increased with increasing added water level as presented in Fig. 4. In the other hand, Fig. 5 shows that the reaction time and enzyme dosage both had significant effect on FFA content, and it increased with increasing reaction time and enzyme dosage.

Figure 6 shows the overly plot for optimization of residual phosphorus and FFA contents. The zone of optimization as shown in the overly plot depicts the reaction time to be in the range of 5.5–7 h, and the temperature between 53 and 60 °C.

Conclusion

Optimum operating parameters of enzymatic degumming process for soybean oil were determined to obtain minimum residual phosphorus and FFA contents. The second-order polynomial model was sufficient to describe and predict the response variable of residual phosphorus and FFA contents to change in the process parameters within the experimental ranges. The graphical optimization method was adopted to find the best enzymatic degumming conditions. Based on current results, the optimum enzymatic degumming parameters within the experimental ranges would be as follow: reaction time of 6.3 h, enzyme dosage of 0.1 g/kg, added water of 2.13 ml/100 g, and temperature of 56 °C. From these optimum results, final residual phosphorus and FFA contents of degummed soy oil were reduced to 10.38 mg/kg and 1.09/100 g using

magnetic immobilized phospholipase A_1. Therefore, it is concluded that magnetic immobilized phospholipase A_1, with a significant thermal stability and reusability, is suitable for the soybean oil degumming process.

Acknowledgments This work was supported by a grant from the National Natural Science Foundation of China (NSFC): Study on method of controlling TFAs in oil by orientated hydrogenation and mechanism of molecular reaction under CO_2 supercritical state (No: 31271886), General program.

Conflict of interest None.

Compliance with Ethics Requirements This article does not contain any studies with human or animal subjects.

References

1. Wim DG, Mare K (2004) Refining practice. In: Hamm W, Hamilton RJ (eds) Edible oil processing. Sheffield Academic Press, UK, pp 83–105
2. Subramanian R, Nakajimaa M, Yasui A, Nabetani H, Kimura T, Maekawa T (1999) Evaluation of surfactant-aided degumming of vegetable oils by membrane technology. J Am Oil Chem Soc 76:1247–1253
3. Dahlke K (1998) An enzymatic process for the physical refining of seed oils. Chem Eng Technol 21:278–281
4. Subramanian R, Raghavarao KSMS, Nabetani H, Nakajimaa M, Kimura T, Maekawa T (2001) Differential permeation of oil constituents in nonporous denser polymeric membranes. J Membrane Sci 187:57–69
5. Clausen K (2001) Enzymatic oil-degumming by a novel microbial phospholipase. Eur J Lipid Sci Technol 103:333–340
6. Klaus D (1998) An enzymatic process for the physical refining of seed oils. Chem Eng Technol 21:278–281
7. Dahlke K, Eichelsbacher M (1998) EnzyMax@ and ALCON@Lurgi's route to physical refining. AOCS Press, Champiagn, pp 53–59
8. De Maria L, Vind J, Oxenboll KM, Svendsen A, Patkar S (2007) Phospholipases and their industrial applications. Appl Microbiol Biotechnol 74:290–300
9. Yang J, Wang Y, Yang B, Mainda G, Guo Y (2006) Degumming of vegetable oil by a new microbial lipase. Food Technol Biotechnol 44:101–104
10. Sheelu G, Kavitha G, Fadnavis N (2008) Efficient immobilization of lecitase in gelatin hydrogel and degumming of rice bran oil using a apinning basket reactor. J Am Oil Chem Soc 85:739–748
11. Yang B, Zhou R, Yang JG, Wang YH, Wang WF (2008) Insight into the enzymatic degumming process of soybean oil. J Am Oil Chem Soc 85:421–425
12. Khoshnevisan K, Bordbar AK (2011) Immobilization of cellulase enzyme on superparamagnetic nanoparticles and determination of its activity and stability. J Chem Eng 171:669–673
13. Bahar T, Celebi SS (2000) Performance of immobilized glucoamylase in a magnetically stabilized fluidized bed reactor (MSFBR). Enz Microb Technol 26:28–33
14. Lei L, Bai Y (2009) Study on immobilization of lipase onto magnetic microspheres with epoxy groups. J Magn Magn Mat 321:252–258
15. Lei L, Liu X (2011) Study on synthesis of poly (GMA)-grafted Fe3O4/SiOX magnetic nanoparticles using atom transfer radical polymerization and their application for lipase immobilization. Mater Chem Phys 125:866–871
16. Liu S, Yang F, Zhang C, Ji H, Hong P, Deng C (2009) Optimization of process parameters for supercritical carbon dioxide extraction of Passiflora seed oil by response surface methodology. J Supercritical Fluids 48:9–14
17. Yu DY, Ma Y, Xue J, Jiang LZ, Shi J (2013) Characterization of immobilized phospholipase A_1 on magnetic nanoparticles for oil degumming application. LWT-Food Sci Technol 50:519–525
18. AOCS (1997) Official methods and recommended practices of the American Oil Chemist's Society. Ca 12-55. AOCS, Champaign
19. AOCS (1997) Official methods and recommended practices of the American Oil Chemist's Society. Ca 5a-40. AOCS, Champaign
20. Yu DY, Jiang LZ, Li ZL, Shi J, Xue J, Kakuda YK (2012) Immobilization of phospholipase A_1 and its application in soybean oil degumming. J Am Oil Chem Soc 89:649–656
21. Bornscheuer U (2000) Enzymes in lipid modification. Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, pp 263–306