Optimization of Enzymatic Degumming Process for Rapeseed Oil

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ABSTRACT: An enzymatic process optimization and a large-scale plant trial for rapeseed oil degumming were carried out by a novel microbial lipase. Response surface methodology was used to obtain the desired data in the process optimization. Enzyme dosage, temperature, and pH were important determining factors affecting oil degumming. The optimal set of variables was an enzyme dosage of 39.6 mg/kg, a temperature of 48.3°C, and a pH of 4.9. The phosphorus content could be reduced to 3.1 mg/kg at the optimal levels of the tested factors. An enzymatic degumming plant trial was performed on a 400 tons/d oil production line. pH was found to play an important role in degumming performance. When the pH was 4.6–5.1, the corresponding phosphorus content of degummed rapeseed oil could be reduced to less than 10 mg/kg, which met the demands of the physical refining process.

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KEY WORDS: Enzymatic degumming, rapeseed oil, response surface methodology.

The purpose of refining vegetable oil is to remove undesirable impurities that affect quality (taste, odor, appearance) and shelf life of the final oil. A number of different refining methods have been developed, and they can be broadly grouped as chemical and physical methods (1–3). Chemical refining of vegetable oils is still the most important and generally practiced method in the oil-refining process. However, use of physical refining is increasing owing to its environmental and economic benefits. Much less wastewater and discharge are produced in physical refining than in the chemical refining and soapstock-splitting processes.

The physical refining route generally consists of a water-degumming step followed by acid degumming, bleaching, and steam-stripping for the purposes of FA removal and deodorization. A prerequisite for the successful process of physical refining is a very low content of phosphatides, i.e., a phosphorus content of less than 15 mg/kg, preferably less than 10 mg/kg. The process is more nearly ideal if the phosphorus content of the oil is less than 5 mg/kg (4). The proper degumming is vital for successful physical refining of vegetable oil. Inadequate degumming will directly influence the efficiency of refining and the quality of consumer-ready oils. However, degumming processes (water degumming, superdegumming, total degumming, ultrafiltration process, acid treatment) cannot always achieve the low phosphorus contents required for physical refining, and they are not always optimally suited for all oil qualities. The refining loss, the cost of the equipment required, and the energy expenditure of these processes are also high (5).

Enzymatic oil degumming is a suitable process for physical refining. It was first developed in the 1990s in initial industrial plant trials by the German Lurgi Company, as the "EnzyMax process" (6). In this process, enzymes change nonhydratable phospholipids into a hydratable form. The EnzyMax process consists of three important steps: adjusting the pH of the oil with buffer, carrying out the enzyme reaction in tanks, and separating gum/sludge from the oil. Every process step is important and must be controlled. Compared with a traditional degumming process, enzymatic degumming has many advantages. In addition to the reduction in the amounts of acid and base used and wastewater generated during the refining process, an enhancement in product yields and a reduction in operating costs can be observed (7).

Efforts to develop other phospholipases to be used for oil degumming have apparently not been successful. Until recently, only two commercial phospholipases, phospholipase A_2 from porcine pancreas and a phospholipase A_1 from *Fusarium oxysporum*, have been in use for oil degumming (8). The porcine pancreatic phospholipases A_2 are not without problems in the application, such as limited enzyme source and the strict pH control required for optimal activity. The current paper reports on a new lipase produced by Novozymes A/S. The enzyme, a microbial lipase from *Thermomyces lanuginosus/F. oxysporum*, exhibits a novel behavior: It displays high specificity for hydrolysis of phosphatides in oil when the reaction temperature is above 40°C (9,10). Thus, it has potential for use in degumming in the oil industry.

EXPERIMENTAL PROCEDURES

The microbial lipase (Lecitase® Ultra; EC 3.1.1.3) was kindly donated by Novozymes A/S (Bagsværd, Denmark). Lipase activity was assayed and found to be 8123 U/g. Two kinds of rapeseed oil were supplied by Southsea Oil & Fat Co. (Shenzhen, China); the phosphorus contents were 212.4 and 120.5 mg/kg, respectively. Polyvinyl alcohol, sodium hydroxide, and citric acid were obtained from Huamei (Shanghai, China). They were of analytical grade and used without further purification. Bleaching earths used in plant trial were obtained from Laiyang Taike Bleaching Agent Co. (Laiyang, China).

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Lipase assay. Enzyme activity was performed with an olive oil emulsion (11). One unit of lipase (U) is the amount of enzyme that releases 1 μmol titratable FFA per minute under the described conditions. The substrate solution, consisting of olive oil and a 4% polyvinyl alcohol solution in a volume ratio of 1:4, was emulsified at 20,000 rpm for 10 min. The citric acid buffer, composed of 48.5 mL of 0.1 M citric acid and 51.50 mL of 0.2 M Na_2HPO_4, was mixed and then diluted to 200 mL with deionized water. In the analysis conditions used, 4 mL of olive oil emulsion, 5 mL of 0.05 M citric acid buffer (pH 5.1), and 1 mL of enzyme solution were mixed and incubated at 40°C for 15 min. The reaction was then terminated by addition of 95% ethanol (15 mL), and the liberated FA were titrated with 0.05 M NaOH. Blanks were measured with a heat-inactivated enzyme sample, for which an enzyme stock solution was kept at 100°C for 15 min. After cooling to ambient temperature, the solution was used as described for the active enzyme sample.

Enzymatic degumming laboratory trial. Crude rapeseed oil (150 g, 212.4 mg/kg phosphate) was placed into a 250-mL conical flask fitted with a stopper. The oil was heated to about 80°C in a water bath, and then 0.2 mL of 45% citric acid was added; the mixture was then homogenized for 1 min at 10,000 rpm. After acidic reaction for 20 min at 80°C with stirring at 500 rpm, the temperature of the oil was decreased to about 50°C, and a suitable amount (0.9–1.3 mL) of 4% NaOH and 3 mL water were added with shear mixing. The enzyme was then added, and the mixture was mixed under high shear (10,000 rpm) for 1 min. The flask was placed in a water bath at 50°C to begin the enzymatic degumming reaction. The oil was stirred at 500 rpm with a mechanical mixer during the entire reaction. After 6 h of incubation, samples were drawn for phosphorus analysis.

Phosphorus content assay. A 10-mL sample of oil emulsion was heated in a boiling water bath for 10 min and then centrifuged at 5000 × *g* for 10 min. The supernatant fluid was collected and mixed, and 8 g of oil was drawn for phosphorus analysis, which was carried out as follows: 100 mg MgO was weighed in a porcelain dish and heated with a gas burner. Oil samples (0.5–2 g) were added and ignited with the gas burner to form a black, hard mass, which was heated at 850°C for 2 h to a white ash. The phosphorus content of the ash was determined according to AOCS method Ca 5a-40 (12).

pH determination. Fifty milliliters of reaction mixture was separated by centrifugation at 8000 × *g* for 10 min. The top oil layer was poured off, and 4 mL of water was added to the gum layer. The gum and water were mixed thoroughly and then centrifuged. After phase separation, the upper oil layer was pipetted off. The pH of the aqueous phase was measured with a pH electrode. To compensate for the dilution effect, measurements were transformed to corrected pH values by the formula: $pH_{corrected} = pH_{measured} - 0.3$. In plant trials, the pH could be directly measured in the heavy phase from the centrifuge.

Experiment design. Response surface methodology was performed to optimize the process of enzymatic oil degumming to obtain the minimum phosphorus content of oil using a central composite rotatable design (CCRD). Enzyme dosage (X_1), pH value (X_2), and temperature (X_3) were identified as the major factors. A set of 15 experiments with three variables were required, with each variable at five levels ($\alpha = 1.68179$). An additional five experiments were performed to verify the accuracy of the model. For statistical calculations, the relation between the coded values and actual values are described by the following equation:

$$X_i = \frac{A_i - A_0}{\Delta A} \quad [1]$$

where X_i = a coded value of the variable; A_i = the actual value of the variable; A_0 = the actual value of A_i at the center point; ΔA = the step change of the variable.

The design matrix is shown in Table 1.The phosphorus content is taken as the response.

The quadratic equation for the variables was the following:

$$Y = \beta_0 + \Sigma\beta_i X_i + \Sigma\beta_{ii} X_i^2 + \Sigma\beta_{ij} X_i X_j \quad [2]$$

where Y = predicted response; β_0 = a constant; β_i = linear coefficient; β_{ii} = squared coefficient; β_{ij} = cross-product coefficient.

Equation 2 was used to build surfaces for the variables. The software SAS 8.02 (Cary, NC) was used to analyze the results. By keeping one variable at its optimal level, 3-D plots of two factors vs. phosphorus content of oil were drawn, and the corresponding contour plot was obtained. From the bump of the 3-D plot or the central point of its respective contour plot, the optimal parameters of enzymatic oil degumming process were identified.

Plant trial. The critical processing parameters were as follows: flow rate of oil, 17 tons/h; flow rate of 45% citric acid, 20 L/h; flow rate of 10% enzyme solution, 8.8 L/h. The total water content was 2–3% of oil. The reaction times were 30 min and 6 h in the citric retention tank and reactor, respectively. The reaction pH was adjusted gradually by controlling the NaOH (4%) addition with a constant flow rate of citric acid, and it was monitored by analyzing the pH of the separated gums online. The enzyme dosage of the reaction retention tank was controlled as 40 mg/kg, and the temperature was 48 ± 2°C. Following the degumming step, physical refining (bleaching and deodorizing) was carried out in a manner similar to the routine refining process. Bleaching earth (1.2%) was used for 20 min, and bleaching occurred at 95°C. After bleaching, the oil was deodorized at 240–250°C for about 2 h until the acid value decreased to about 0.1% or less.

RESULTS AND DISCUSSION

Lecitase Ultra is an acidic lipase that exhibits maximal activity at pH 5.0. It has inherent activity toward both phospholipid and TG structures. Its phospholipase activity predominates when the temperature is over 40°C, and its maximum is exhibited at 50°C. When citric acid buffer was added to the oil and mixed at 80°C for 20 min, the phosphorus content of the crude oil decreased from 212.4 to 33.5 mg/kg. After the reaction mixture had cooled to 50°C, NaOH solution, water, and enzyme solution

TABLE 1
Central Composite Rotatable Design with Three Variables and Five Levels[a]: Experimental Design and Responses for the Phosphorus Content in the Oil

	Coded values and real values			Phosphorus content (mg/kg)	
Run	X_1 (mg/kg)	X_2 (pH)	X_3 (°C)	Actual values	Predicted values
1	–1 (20)	–1 (4.7)	–1 (45)	13.8	13.8
2	–1 (20)	–1 (4.7)	1 (51)	12.4	13.0
3	–1 (20)	1 (5.3)	–1 (45)	15.2	15.2
4	–1 (20)	1 (5.3)	1 (51)	14.5	15.2
5	1 (40)	–1 (4.7)	–1 (45)	5.9	5.4
6	1 (40)	–1 (4.7)	1 (51)	4.6	4.8
7	1 (40)	1 (5.3)	–1 (45)	8.0	7.6
8	1 (40)	1 (5.3)	1 (51)	7.5	7.7
9	–1.6818 (13)	0 (5.0)	0 (48)	18.3	17.7
10	1.6818 (47)	0 (5.0)	0 (48)	4.1	4.4
11	0 (30)	–1.6818 (4.5)	0 (48)	9.7	9.6
12	0 (30)	1.6818 (5.5)	0 (48)	13.5	13.3
13	0 (30)	0 (5.0)	–1.6818 (43)	7.3	7.9
14	0 (30)	0 (5.0)	1.6818 (53)	8.2	7.3
15	0 (30)	0 (5.0)	0 (48)	5.2	5.2
16	0 (30)	0 (5.0)	0 (48)	5.5	5.2
17	0 (30)	0(5.0)	0 (48)	5.1	5.2
18	0 (30)	0 (5.0)	0 (48)	5.0	5.2
19	0 (30)	0 (5.0)	0 (48)	5.0	5.2
20	0 (30)	0 (5.0)	0 (48)	5.2	5.2

[a]X_1 = enzyme dosage, X_2 = pH, X_3 = temperature. Real values are given in parentheses.

(30 mg/kg of enzyme) were added, and the oil was degummed to less than 10 mg/kg of phosphorus within 6 h, whereas the residual phosphorus was about 20 mg/kg if no enzyme was added. Further experiments were performed to optimize the conditions for oil degumming by the microbial lipase.

Estimation of the coefficients in a model design. A CCRD was used to identify the importance of the variables (enzyme dosage, pH value, and temperature) in the oil-degumming process. Twenty treatments were established using a computer simulation with Equation 1 (Table 1). Each treatment was performed to reduce the phosphorus content in the oil. Treatments 15–20 in the experiment were done under the same conditions. The results suggested accuracy of our experiments.

Model analysis to estimate the coefficients. SAS 8.02 software was used to determine a quadratic mathematical model of degumming. By applying multiple regression analysis to the experimental data (Table 1), the following second-order polynomial equation was generated, representing the phosphorus content in the oil as a function of variables:

$$Y = 918.9636 - 2.014499X_1 - 255.1006X_2 - 10.31671X_3 + 0.020803X_1^2 + 0.0625X_1X_2 + 0.00125X_1X_3 + 24.68601X_2^2 + 0.208333X_2X_3 + 0.095618X_3^2 \quad [3]$$

where Y is the predicted response and X_1, X_2, and X_3 are the uncoded values (enzyme dosage, pH, and temperature, respectively).

The significance of each coefficient was determined by Student's *t*-test and *P*-values. Table 2 lists the regression coefficients calculated by the model for β_i, β_{ii}, and β_{ij} along with significance levels of the terms. From *P*-values for terms in Table 2, one can seen that the linear term for temperature had the least significance with respect to the phosphorus content of the oil. The linear and square terms of pH and enzyme dosage were

TABLE 2
Estimate for Effect of Phosphorus Content in the Oil[a]

Term	Coefficient	SE	*t*	Pr[b] > |*t*|
X_1	–3.9381	0.1496	–26.3277	0.0001***
X_2	1.0904	0.1496	7.2895	0.0001***
X_3	–0.1747	0.1496	–1.1682	0.2698
X_1X_1	2.0803	0.1456	14.2869	0.0001***
X_1X_2	0.1875	0.1954	0.9594	0.3600
X_1X_3	0.0375	0.1954	0.1919	0.8517
X_2X_2	2.2217	0.1456	15.2581	0.0001***
X_2X_3	0.1875	0.1954	0.9594	0.3600
X_3X_3	0.8606	0.1456	5.9100	0.0001***

[a]X_1, enzyme dosage; X_2, pH; X_3, temperature.
[b]***Statistically significant at 99% confidence level.

TABLE 3
Analysis of Variance and Regression for the Phosphorus Content in the Oil

Source	Degrees of freedom	Sum of squares	Mean square	*F*–value	Pr > *F*
Model error	9	353.7645	39.3072	128.64	0.0001***
Error	10	3.055543	0.3056		
Total	19	356.82			
Coefficient of correlation (R^2), 99.14%					
Coefficient of determination (adjusted R^2), 98.37%					
CV, 6.353675%					

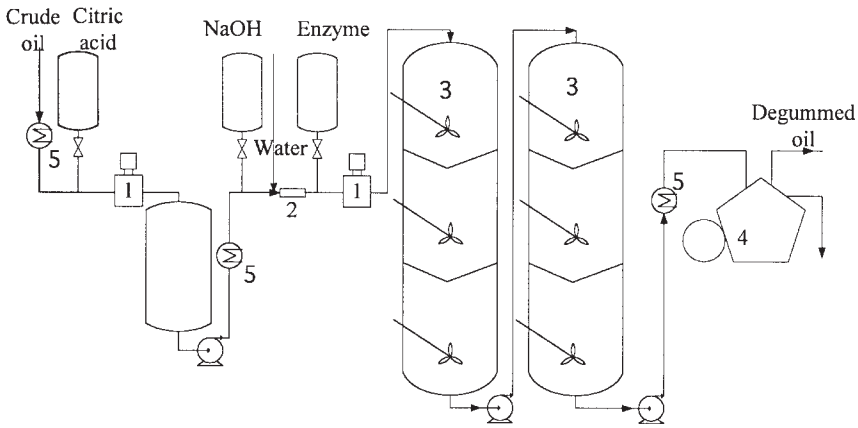

FIG. 1. Flow diagram of the oil-degumming process.

highly significant, and the square term of temperature was also one of the most influential factors. The interaction of pH and enzyme dosage and temperature exerted only a minor effect on the phosphorus content of the degummed oil.

The statistical significance of the second-order model equation was evaluated by the *F*-test ANOVA (Table 3). The mathematical model was very reliable, with an R^2 value of 99.14%. The closer R^2 is to 1, the better the model fits the experimental data, and the less the difference between the predicted and the observed values. The low CV value of 6.35% indicated a great degree of precision with which the treatments were compared. The computed *F*-value of 128.64 was greater than the $F_{(9,10)}$ value in statistical tables at a 1% level. It reflected the significance of the model.

Optimization of enzymatic process parameters of rapeseed oil. The response surface curves and the contour curves for enzyme dosage, pH, and temperature are shown in Figures 1–3. The response surface representing residual phosphorus content in the oil was a function of two factors with another variable held at an optimal level. The optimal levels and interaction between two factors were clearly revealed by the study. In the experimental design, central points were fixed by prior experiments (9,10).

The residual phosphorus content in the degummed oil decreased with increasing enzyme dosage (Figs. 2, 4). The lowest phosphorus content was achieved at pH 4.9 and 48°C (Fig. 3). Lower and higher values of pH and temperature reduced the removal of phosphorus from oil (Figs. 2–4).

According to the mathematical model, the optimal levels of

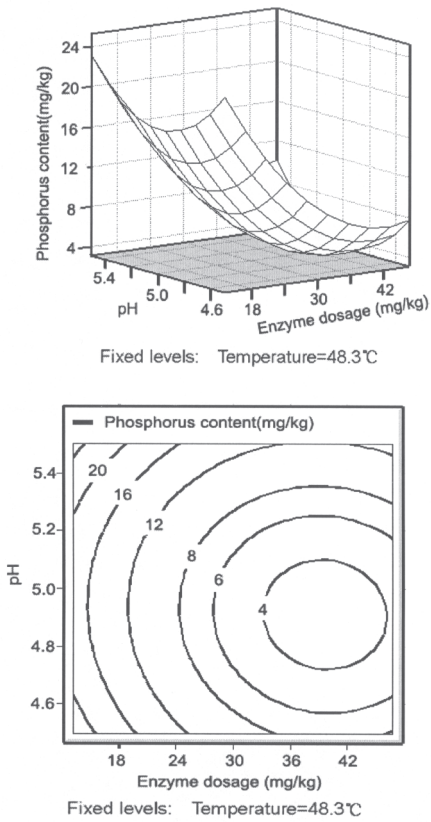

FIG. 2. Response surface plot (upper) and its contour plot of resident phosphorus content in the oil; pH vs. enzyme dosage (lower) with constant temperature (48.3°C).

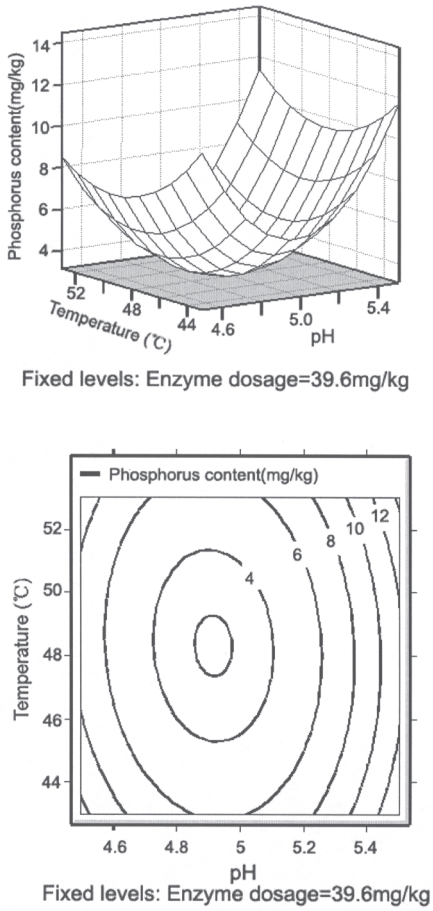

FIG. 3. Response surface plot (upper) and its contour plot of resident phosphorus content in the oil; temperature vs. pH (lower) with constant level of enzyme dosage (39.6mg/kg).

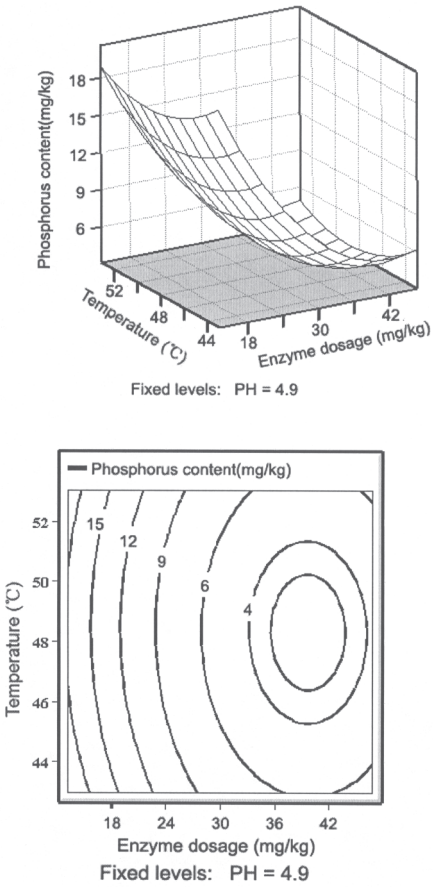

FIG. 4. Response surface plot (upper) and its contour plot of resident phosphorus content in the oil; Temperature vs. enzyme dosage (lower) with constant level of pH (4.9).

the three factors were: enzyme dosage 39.6 mg/kg, pH 4.9, and temperature 48.3°C; the corresponding residual phosphorus content was 3.1 mg/kg. The optimal values obtained from response surface plots were consistent with those obtained from the optimized mathematical equation. To verify the predicted results, the experiment was performed under optimized conditions. Oil degummed under these conditions contained 3.3 mg phosphorus/kg oil, which was comparable to that of the predicted values of the residual phosphorus.

Plant trial. Based on the optimum conditions, the enzymatic degumming trial was carried out on a 400 ton/d production line (Fig. 1). For this capacity the reactor was designed as two towers in series with three stages in each tower. Thus, the reactor was a series of six continuous stirred tank reactors. The results are shown in Figure 5.

The phosphorus content of oil in the first stage of the reactor was 63.3 mg/kg, which was already substantially lower than the phosphorus content of the crude oil (120.5 mg/kg) because of coagulation and precipitation of part of the phosphatides caused by addition of citric acid. The pH had a strong impact on the degumming performance (Fig. 5). The phosphorus content of the oil decreased with a lowering of pH in the first stage, and less than 10 mg/kg could easily be obtained when the pH was kept in the range of 4.6–5.1. The phosphorus content could be further lowered to about 3 ppm after the bleaching process;

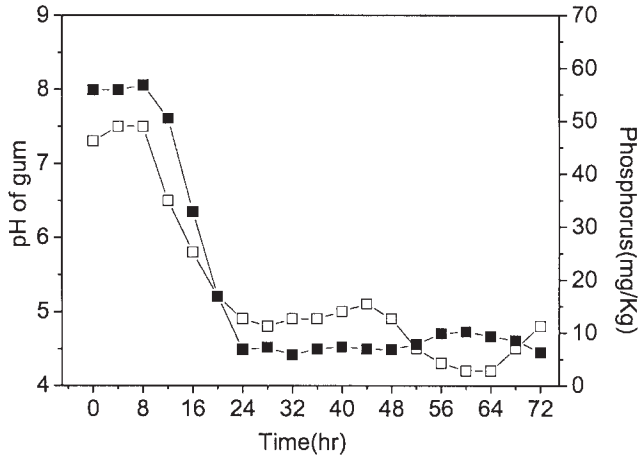

FIG. 5. Results from industrial trial for rapeseed oil degumming. (□) pH of gum; (■) phosphorus content of the oil.

3 ppm is generally low enough for the physical refining process. However, the bleaching earth consumption was a little higher than for the chemical refining process. After conventional deodorization of the oil, the phosphorus content was about 1–2 ppm, which was similar to the chemically refined oils.

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