Effect of enzymatic treatment on rapeseed oil degumming and its quality

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Abstract

An experiment of rapeseed oil degumming by a phospholipase A_1 (*Lecitase® Ultra*) had been carried out for the study of effect of enzymatic treatment on the rapeseed oil degumming and its quality. The experimental results show that the *Lecitase® Ultra* plays an important role in the degumming of rapeseed oil. By doing orthogonal experiment, the optimum conditions of rapeseed oil degumming by a phospholipase A_1 were obtained as follows: pH 4.82, dosage of *Lecitase® Ultra* 150LU/kg, temperature 37°C and time of enzymatic treatment 3h. Under these conditions, the phosphorus content in obtained degummed rapeseed oil was 6.97mg/kg. At the same time, the acid value and peroxide value of the degummed oil increased, and its color had a beneficial change. In the degummed rapeseed oil, the contents of palmitic acid and oleic acid decreased, but the contents of linolenic acid, linoleic acid, eicosenoic acid and erucic acid increased, and the content of other fatty acids didn't evidently change.

Key words: Phospholipase, Rapeseed Oil,Degumming, Quality

Introduction

Enzymatic oil-degumming is a suitable process for physical refining, in which the non-hydrateable phosphatide is converted, by using a kind of phospholipase hydrolyses the ester bonds of the phospholipids in the crude oil, into a water-soluble lyso-phosphatide and is separated as a heavy phase (Klaus Dahlke, 1998). Until now, only two commercial phospholipases, which are phospholipase A2 from porcine pancreas and a phospholipase A1 from Fusarium oxysporum, are in practice for oil-degumming (Bruno H. Winter, et al., 1998; Kim Clausen, 2001). In Fig. 1, phospholipid hydrolyzing by phospholipase is shown, the non-hydrateable phosphatides consist to a high extent of phosphatidic acid, i.e., the remaining X at the phosphate group is simply a hydrogen. The lyso-phosphatide, produced in enzymatic degumming reaction, exhibits a hydrophilic group, consisting of the phosphate group and the hydroxyl group at the 1 or 2-position.

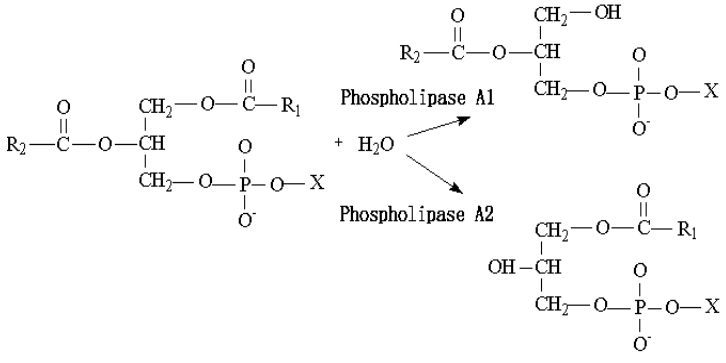

Fig.1 Phospholipid hydrolyzing by phospholipase

A generalized depiction of a phospholipid, where X = H, choline, ethanolamine, inositol, etc.

Enzymatic oil-degumming was firstly developed in the 1990s with the initial industrial plant trials by the German Lurgi company (Klaus Dahlke, 1998). This new oil-degumming process was called the "EnzyMax process", in which the phospholipase A_2 from porcine pancreas was used to change nonhydratable phospholipids into a hydratable form. Comparing with traditional degumming process, enzymatic degumming has many advantages. Apart from the reduction in the amount of acid, base and waste water during refining process, an enhancement in product yields and a reduction in operating costs can also be observed (Klaus Dahlke, 1998; Bruno H. Winter, et al., 1998; Kim Clausen, 2001; Roy S.K., et al., 2002; Ji-Guo Yang, et al., 2006). Because the porcine pancreatic phospholipase A_2 meet some problems in the application, such as limited source, expensive price and defect in function, "EnzyMax process" has not been popularized in large scale. Recently, it is found that the phospholipase A_1 from *Fusarium oxysporum* can be used in oil-degumming process(Roy S.K., et al., 2002). The phospholipase A_1 can be largely produced by fermentation, furthermore some new and excellent performance phospholipase can be obtained by filtrating, which makes enzymatic oil-degumming more economy and availability in practice.

Lecitase® Ultra is a new microbial lipase developed by Novozymes, and it is a protein-engineered carboxylic ester hydrolase from *Thermomyces lanuginosus/ Fusarium oxysporum* produced by submerged fermentation of a genetically

modified *Aspergillus oryzae*. The aim of this work was to study the oil-degumming process using *Lecitase® Ultra*, and evaluate the effect of enzymatic treatment on the rapeseed oil quality.

2 Materials and Methods

2.1 Materials

Water-degummed rapeseed oils were supplied by Zhongpai Grain & Oil Ltd (Wuhan, China), and the phosphorus content typically was 177.62mg/kg.

Phospholipase A_1 (Lecitase® Ultra, E.C 3.1.1.3) was kindly donated by Novozymes A/S, Bagsvaerd, Denmark.

Sodium hydroxide and *citric acid* were obtained from Tianda (Tianjin, China). They were of analytical grade and used without further purification.

2.2 Methods

2.2.1 Enzymatic degumming lab trial

Water-degummed rapeseed oil (100 g, 177.62mg/kg phosphate) was placed into a 250 mL conical flask fitted with stopper. The oil was heated to about 80 °C in a water bath, and then 0.16 mL of 45 % citric acid was added and homogenized for 1.5 min at 8000 rpm. After acidic reaction for 20min at 80°C under 300 rpm, the temperature of the oil was decreased to temperature preset, and a suitable amount of 4 % NaOH were added with shear mixing at 300 rpm for 5 min. After reaction completion, enzyme and water were added with shear mixing. The flask was placed in a water bath of preset temperature to begin enzymatic degumming reaction with shear mixing at 500 rpm. After certain long time incubation, oil emulsion was heated in 80°C for 10 min and then centrifuged at 4800 rpm for 20 min. After phase separation, top layer oils were collected in an airtight container for phosphorus analysis.

2.2.2 Phosphorus content analysis

Phosphorus analysis was carried out as follows: 100 mg of MgO were weighed in a porcelain dish and heated on a gas burner. A mass of 0.5–2 g of oil was added and ignited with a gas burner to become a black, hard mass, and then it was heated at 850 °C for 2 h until it turned into white ash. The phosphorus content of the ash was determined according to AOCS method Ca 12–55. All experiments were carried out in triplicate for the calculation of the mean value.

2.2.3 pH determination(Kim Clausen, 2001)

5 ml of water in oil emulsion were mixed with 5ml of MilliQ water. And then mixtures were separated by centrifugation at 4800rpm for 20min. After phase separation, top layer oil was pipetted off. The pH in aqueous phase was measured with pH electrode Mettler-Toledo. In order to compensate for the dilution effect, measurements were transformed to corrected pH values by formula ($pH_{corrected} = pH_{measured} - 0.38$).

3 Results and Discussion

3.1 Single-factor experiments

3.1.1 Effect of enzymatic treatment time on degumming of rapeseed oil

Some enzymatic degumming trials were done by way of method 2.2.1 mentioned, and the reaction conditions were controlled as follow: pH 4.82, dosage of *Lecitase® Ultra* 300LU/kg, temperature 48°C, 0.55ml of 4% NaOH, total water content 2.5% and time of enzymatic treatment 0, 1, 2, 3, 4, 5h, respectively. After degumming process was completed, the phosphorus content in the examples of degummed oil was analyzed respectively and the determined results were displayed in Fig.2.

As shown in Fig.2, within 1h of the beginning of enzymatic degumming reaction, the phosphorus content decreased very rapidly from 177.62 mg/kg to 46.08 mg/kg. The addition of citric acid buffer and *Lecitase® Ultra* had already caused together coagulation and precipitation of part of phosphatides. Therefore, the phosphorus content in the oil was substantially lower than the phosphorus content of Water-degummed oil after the acid treatment. After enzymatic degumming reaction went on for 1h, *Lecitase® Ultra* became a main factor for removal of nonhydratable phospholipids. As the result, the speed of phosphorus content decreasing became slower. Beyond 3h of the enzymatic degumming reaction, addition of reaction time could almost not reduce phosphorus content of oil. Thought of saving production time and increasing degumming efficiency, 3h was an appropriate time for this enzymatic degumming reaction.

3.1.2 Effect of pH on degumming of rapeseed oil

The degumming tests were done by way of method mentioned above, and the reaction conditions were controlled as follow: dosage of *Lecitase® Ultra* 150LU/kg, temperature 48°C, total water content 2.5% and time of enzymatic treatment 3h, and pH 4.13, 4.42, 4.65, 4.82, 4.97, 5.15, 5.39, respectively. After degumming process was completed, the phosphorus content in the examples of degummed oil was analyzed respectively and the results were shown in Fig.3.

The pH optimum of this enzyme for degumming of rapeseed oil was displayed in Fig.3. *Lecitase® Ultra* is an acidic lipase, which pH has a remarkable effect on its catalytic activity. As shown in Fig.3, *Lecitase Ultra* exhibits maximal degumming performance at pH 4.82.

3.1.3 Effect of dosage of *Lecitase® Ultra* on degumming of rapeseed oil

The enzymatic degumming reaction conditions were controlled as follow: temperature 48°C, 0.6ml of 4% NaOH, total water content 2.5% and time of enzymatic treatment 3h, and dosage of *Lecitase® Ultra* 50, 100, 150, 225, 300, 375, 450LU/kg respectively. The phosphorus content in the examples of degummed oil was analyzed respectively and the results were

displayed in Fig.4.

As shown in Fig.4, within 150LU/kg of enzymatic dosage, dosage of enzyme played an important role in effect of degumming. Beyond 150LU/kg of enzymatic dosage, addition of enzymatic dosage reduced hardly phosphorus content in the example of degummed oil. Thus, thought about economy of production, 150LU/kg was an appropriate of enzymatic dosage for rapeseed oil degumming.

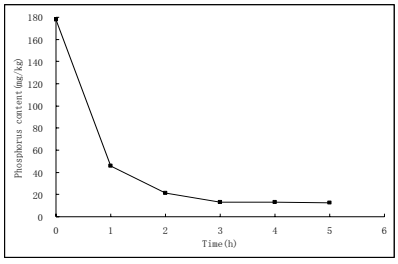

Fig.2 Effect of enzymatic treatment time on phosphorus content of degummed rapeseed oil

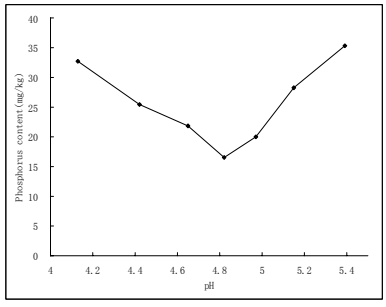

Fig.3 Effect of pH on phosphorus content of degummed rapeseed oil

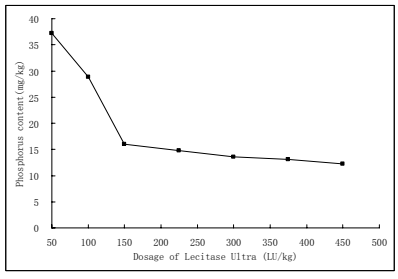

Fig.4 Effect of dosage of *Lecitase® Ultra* on phosphorus content of degummed rapeseed oil

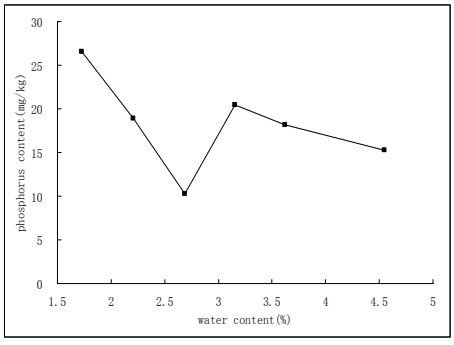

Fig.5 Effect of content of water on phosphorus content of degummed rapeseed oil

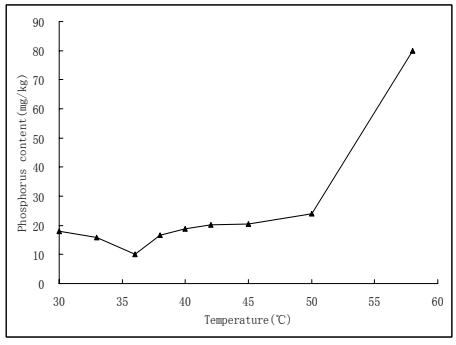

Fig.6 Effect of temperature on phosphorus content of degummed rapeseed oil

3.1.4 Effect of content of water on degumming of rapeseed oil

The enzymatic degumming reaction conditions were controlled as follow: dosage of *Lecitase Ultra* 150LU/kg, temperature 48°C, 0.6ml of 4% NaOH, time of enzymatic treatment 3h, and total water content 1.73%, 2.21%, 2.69%, 3.16%, 3.62%, 4.54%, respectively. After enzymatic degumming process was finished, the phosphorus content in the example of degummed oil was analyzed respectively and the determined results were displayed in Fig.5.

As shown in Fig.5, when total water content varied from 1.73 to 2.69% in the oil-degumming experiments, the more water was added, the better the enzymatic degumming effect was. However, when the total water content was between 2.69% and 3.16%, the phosphorus content of degummed rapeseed oil increased correspondingly. Within 3.16%~4.54% of total water content, the phosphorus content of degummed rapeseed oil reduced slowly. *Lecitase® Ultra* has ability to perform oil-degumming at reduced water content as shown in Fig. 5. At total water content 2.69%, it is still possible to degum to a final phosphorus level at 10 ppm within 3h. Therefore, the optimum water content was 2.69%.

3.1.5 Effect of temperature on degumming of rapeseed oil

The enzymatic degumming reaction conditions were controlled as follow: 0.6ml of 4% NaOH, total water content 2.69%, time of enzymatic treatment 3h, and dosage of *Lecitase® Ultra* 150LU/kg, and temperature 30, 33, 36, 38, 40, 42, 45, 50, 58°C, respectively. The phosphorus content in the example of degummed oil was analyzed respectively and the results were displayed in Fig.6.

Temperature is an important effect factor for enzymatic catalytic reaction. As shown in Fig.6, *Lecitase® Ultra* had a better catalytic activity at normal temperature. When reaction temperature varied between 30°C and 45°C, the phosphorus content of degummed rapeseed oil changed little, and *Lecitase® Ultra* had a best effect of degumming for rapeseed at 38°C. When reaction temperature was increased over 50°C, *Lecitase® Ultra* catalytic activity reduced quickly and phosphorus content of degummed rapeseed oil increased rapidly.

3.2 Optimized experiment

Referenced results of single factor experiments, pH, dosage of *Lecitase® Ultra* and temperature were chosen as important factors and using $L_9(3)^4$ model designed an orthogonal experiment for optimizing enzymatic degumming process. Factors and levels in the experiment of degumming by enzyme were shown in table 1.

With phosphorus content of degummed rapeseed oil as evaluation index, total water content 2.69% and enzymatic reaction time 3h, orthogonal experiments were done, and then experiments results were shown in table 1.

Table 1 Result of orthogonal experiment

Test number	pH	Dosage of *Lecitase Ultra* LU/kg	Temperature °C	Empty List	Phosphorus content mg/kg
1	4.65	150	33	1	15.74
2	4.65	225	37	2	12.39
3	4.65	300	41	3	14.26
4	4.82	150	37	3	6.97
5	4.82	225	41	1	9.26
6	4.82	300	33	2	8.03
7	5.06	150	41	2	18.22
8	5.06	225	33	3	13.35
9	5.06	300	37	1	11.59
Average 1	14.13	13.64	12.37	12.20	
Average 2	8.09	11.66	10.32	12.88	
Average 3	14.38	11.29	13.91	11.53	
R	6.29	2.35	3.59	1.35	

From results of range analysis showed in table 1, it was seen that sequence, three factors effect on enzymatic degumming, as follow: pH>temperature> dosage of *Lecitase® Ultra.* As shown in table 2, a conclusion could be made that pH have a remarkable effect on degumming rapeseed oil, but effect of temperature and dosage of *Lecitase® Ultra* on degumming rapeseed oil were inconspicuous. Through analysis of range for the results of orthogonal experiment, the optimum conditions of rapeseed oil degumming by *Lecitase® Ultra* were obtained as follows: pH 4.82, dosage of *Lecitase® Ultra* 300LU/kg, temperature 37°C and time of enzymatic treatment 3h. On these conditions, the phosphorus content in obtained degummed rapeseed oil was 6.32mg/kg. Contrast with No. 4 of table 1, phosphorus content induced 0.65 mg/kg, but dosage of *Lecitase® Ultra* doubled. Thus, thought of products cost, The reaction conditions of No. 4 were determined as optimum parameter of rapeseed oil degumming by *Lecitase® Ultra*.

Table 2 Analysis of variance for the result of orthogonal experiment

Variation source	Quadratic sum of deviation	Degree of freedom	F-ratio	$F_{\alpha=0.05}$	Significance
pH	76.294	2	27.967	19	*
Dosage of *Lecitase Ultra* LU/kg	9.599	2	3.519	19	
Temperature°C	19.507	2	7.151	19	
Empty List	2.728	2	1	19	
Error	2.73	2			

3.3 Effect of enzymatic treatment on quality of the rapeseed oil

3.3.1 Effect on acid value of the oil

Acid value of nine kinds of oils examples, which chosen from rapeseed oil degummed by enzyme at random, were determined and contrast with water-degummed rapeseed oil (Fig.7). As shown in Fig. 7, after enzymatic degumming process, acid value of degummed oil had a certain degree rise and the addition range was between 0.28 mgKOH/g and 1.04 mgKOH/g, most acid value of degummed oil were under 3.00mgKOH/g.

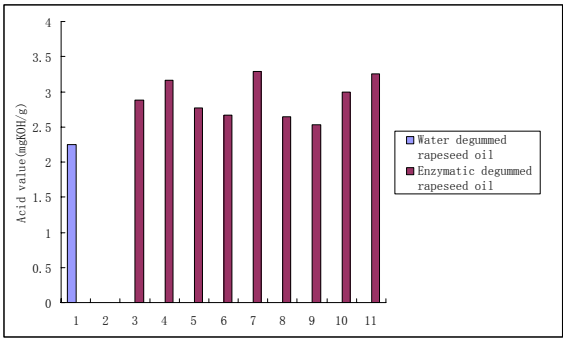

Fig.7 Effect of enzymatic treatment degumming on acid value of rapeseed oil

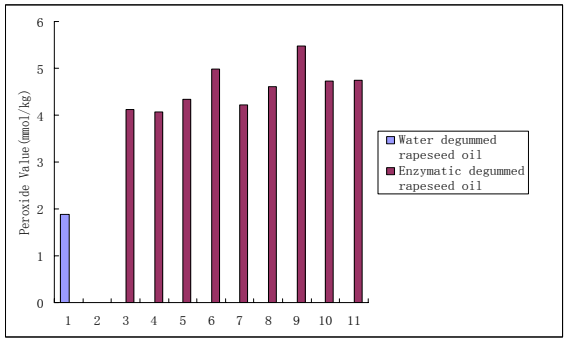

Fig.8 Effect of enzymatic treatment degumming on peroxide value of rapeseed oil

It is well known that one fatty acid will be removed from one phospholipid molecule in the enzymatic degumming process. As a result, there will raise content of the free fatty acids (FFA). In this study, it was found that the amount of FFA increase fitted well to that of the decrease of phosphorus. Therefore, it was concluded that the increase of free fatty acids was the consequence of the hydrolysis of phospholipids rather than triglycerides. It was an interesting phenomenon that the *Lecitase® Ultra* enzyme was able to identify only the phospholipids as substrate, and did not hydrolyze the triglyceride in the

enzymatic oil degumming system, although the lipase activity of the *Lecitase® Ultra* enzyme was remarkable in the other system (Ji-Guo Yang, et al., 2006). The mechanism of this enzymatic reaction needs to be explored in the future research.

3.3.2 Effect on Peroxide Value of the oil

Peroxide value of nine kinds of oils examples, which chosen from rapeseed oil degummed by enzyme at random, were determined and contrast with water-degummed rapeseed oil (Fig.8). As shown in Fig. 8, after enzymatic degumming process, peroxide value of degummed oil had a certain degree rise, and most scales reach 3.60 mmol/kg. However, all of peroxide value of nine kinds of oils examples below 6.00 mmol/kg, most were between 4.00 and 5.00 mmol/kg.

3.3.3 Effect on fatty acids composition of the oil

The colors of oils were determined by Lovibond Tintometer with 2.54 cm cell. As shown in table 3, the color of enzymatic degummed oil was better than water degummed oil. And a conclusion could be made that color of rapeseed oil became thinness by enzymatic degumming. The content of palmitic acid and oleic acid decreased, but the content of linolenic acid, linoleic acid, eicosenoic acid and erucic acid increased, and other the content of fatty acid didn't evidently change (table 3).

Table 3 Effect of enzymatic treatment degumming on color and fatty acids composition of rapeseed oil

Kind of oil / Test Items	Water degummed rapeseed oil	Enzymatic degummed rapeseed oil
Color (Lovibond Tintometer with 2.54 cm cell)	Y35 R4.6	Y35 R4.0
Myristic Acid %	—	0.1
Palmitic acid %	4	3.3
Palmitoleic Acid %	0.3	0.3
Heptadecanoic Acid %	0.1	0.1
Stearic acid %	1.7	1.6
Oleic acid %	38.6	33.7
Linoleic acid %	14.4	14.7
Linolenic acid %	6.5	7.1
Arachidic Acid %	0.9	0.8
Eicosenoic Acid %	7.8	8.6
Eicosadienoic Acid %	0.4	0.3
Docosanoic Acid %	0.5	0.5
Erucic Acid %	25.1	29

4 Conclusions

4.1 The optimum conditions of rapeseed oil degumming by a phospholipase A_1 (*Lecitase® Ultra*) were obtained as follows: pH 4.82, dosage of *Lecitase® Ultra* 150LU/kg, temperature 37°C and time of enzymatic treatment 3h. On these conditions, the phosphorus content in obtained degummed rapeseed oil was 6.97mg/kg. pH have a remarkable effect on degumming rapeseed oil by *Lecitase® Ultra*, but effect of temperature and dosage of *Lecitase® Ultra* on degumming rapeseed oil was inconspicuous.

4.2 After rapeseed oils were degummed by enzyme, the acid value and peroxide value of the degummed oil rose, the most increased value was 1.04mgKOH/g and 3.60 mmol/kg, respectively. And its color had a beneficial change. In degummed rapeseed oil, the content of palmitic acid and oleic acid decreased, but the content of linolenic acid, linoleic acid, eicosenoic acid and erucic acid increased, and other the content of fatty acid didn't evidently change.

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