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ORIGINAL PAPER

Insight into the Enzymatic Degumming Process of Soybean Oil

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Abstract An enzymatic degumming trial of soybean oil was carried out at a capacity of 400 tons/day by applying microbial phospholipase A_1 from *Thermomyces lanuginosus/Fusarium oxysporum*. When the pH was kept in the range of 4.8–5.1, less than 10 mg/kg of phosphorous content of The oil was obtained. The gum and oil were easily separated after centrifugation and the oil loss was minimal under the process conditions. Through analysis of phospholipids compounds in the gum by Electrospray Ionization-Mass Spectrometer and phosphorous content, it could be seen that both glycerophospholipids and lysophospholipids existed with contents of 45.7 and 54.3%, respectively. The performance of enzymatic degumming was found to be related to the production of glycerophospholipids.

Keywords Enzymatic degumming · Phospholipase · Soybean oil

Introduction

Vegetable oils, such as oils from soybean, sunflower and rapeseed, must be refined to remove the impurities in order for them to be suitable for direct human consumption. The refining of edible oils and fats can be carried out using two routes, namely chemical and physical refining. In the former process, more wastewater and discharge are produced and higher refining losses are caused especially for oils with high free fatty acid content. Physical refining on the other hand is more attractive because of its environmental and economic benefits. Practical experience, however, shows that low phosphorous content are of the utmost importance for successful physical refining. It is ideal if the phosphorous content in the oil is less than 5 mg/kg [1]. Therefore, the proper degumming process is the crucial basis for the successful physical refining of vegetable oils.

Many degumming methods have been designed for physical refining, including water degumming [2], superdegumming [3], total degumming [4], an ultrafiltration process [5] and acid treatment [6]. Acid degumming is a widely used method, by which phosphorus or citric acids are used and the phosphorus content after degumming is about 15–80 mg/kg depending on the different oil resources and quality. Though acid degumming is a very simple method, it is not sufficiently reliable for all types of oils. Superdegumming and total degumming, regarded as modified processes of acid degumming, are seldom used in oil plants because of their unreliability. The ultrafiltration process is still a new method in the oil industry as membrane fouling, which will lead to a poor economic performance, is still a serious problem. Up to now, there is still no good degumming method that is qualified for the physical refining for all oils irrespective of their initial quality [7].

Enzymatic degumming was firstly reported in the 1990s by Roehm and Lurgi concerning the commercial "Enzy-Max process" [8], in which enzyme was used to hydrolyze nonhydratable phospholipids into their hydratable form. The enzymatic oil-degumming process is shown in Fig. 1. In comparison to traditional degumming processes, an

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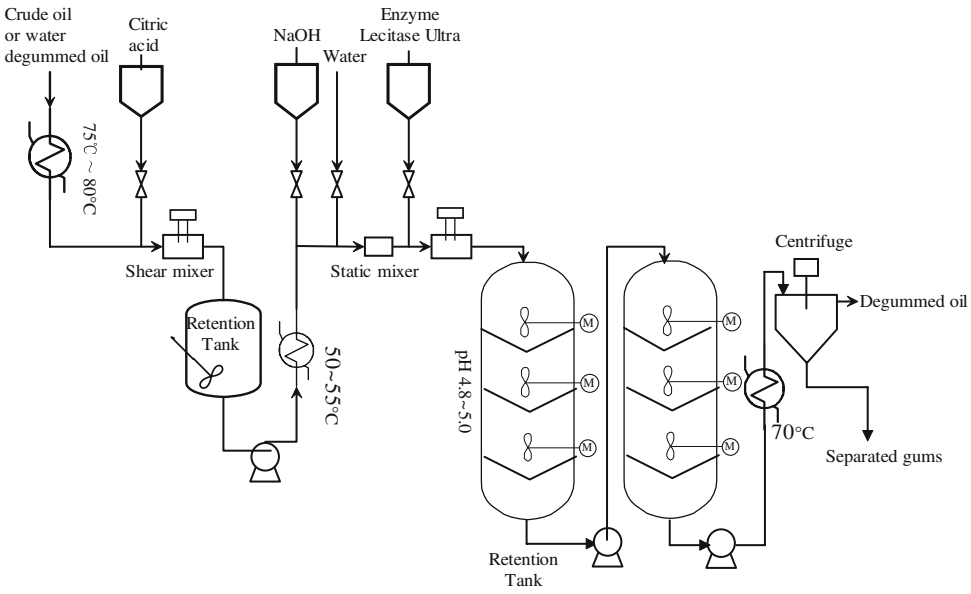

Fig. 1 Flow diagram of the oil-degumming process

enhancement in product yields and a reduction in wastewater and operating costs were observed.

Enzymatic degumming processes have been explored by other researchers with phospholipases from different sources [9]. Under suitable conditions in a small scale process, a low phosphorous content (<10–15 ppm) was obtained. For successful industrial applications, however, only three enzymes, i.e. a phospholipase A_2 from porcine pancreas and two kinds of microbial phospholipase A_1 from *Fusarium oxysporum* and *Thermomyces lanuginosus/Fusarium oxysporum*, were available for enzymatic oil-degumming [10]. Until recently, work on enzymatic oil-degumming was mainly focused on the development of new enzymes and on the optimal processes for the reduction of phosphorous content.

In this paper we report the application of microbial phospholipase A_1 from *Thermomyces lanuginosus/Fusarium oxysporum* for the degumming of soybean oil at 400 tons/day oil line and describe the enzymatic process and analysis of the phospholipids composition of the gums as well as discuss the degumming mechanisms.

Experimental Procedures

The microbial lipase (phospholipase A_1, Lecitase® Ultra, E.C 3.1.1.3) was kindly donated by Novozymes A/S, Bagsvaerd, Denmark. Phospholipase activity was found to be 8123 U/g. Soybean oil was supplied by the Southsea Oil & Fat Co. (Shenzhen, China), with a phosphorus content of 121.5 mg/kg. Polyvinyl alcohol, sodium hydroxide and citric acid, obtained from Huamei (Shanghai, China), were of analytical grade and used without further purification.

Lipase assay. Enzyme activity was performed with an olive oil emulsion according to the published procedure [11]. One unit of lipase (U) is the amount of enzyme that releases 1 μmol titratable free fatty acids per minute under the described conditions.

Plant trial. A large-scale trial of soybean oil-degumming was performed at the 400 tons/day line. The reactor and process (Fig. 1) is a series of six continuous stirred tank reactors and 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 time was 30 min and 6 h in the citric retention tank and reactor, respectively. The reaction pH was adjusted gradually by controlling the addition of NaOH (4%) with a constant flow rate of citric acid, and it was monitored by analyzing the pH of the separated gums on line. The enzyme dosage of the reaction retention tank was controlled at 40 mg/kg, and the temperature was 48 ± 2 °C. The gum was separated continuously at 10,000*g* by one self-cleaning centrifuge made by Westfalia. After degumming, the bleaching process was run as routine operation with 1.2% bleaching earth dosage. The temperature of physical deodorization was controlled as 245 ± 3 °C.

pH determination. The pH of gum could be directly measured using the gum from the centrifuge in plant trial. In order to compensate for the dilution effect, measurements were transformed to corrected pH values by the following formula: $pH_{corrected} = pH_{measured} - 0.3$.

Pretreatment of gum. The gum was dried by rotary evaporation, and a residue was obtained. Ten grams of the residue was withdrawn and placed into a 50-ml glass tube, and then 25 ml toluene was added and mixed sufficiently

by swirling for 10 min. The mixture was centrifuged at 15,000*g* for 5 min, and the upper phase was removed to another tube. The sediment was extracted twice again with toluene and the upper phase collected and dried by rotary evaporation. The sediment that was insoluble in toluene was referred to as A, and the materials soluble in toluene referred to as B. Material A and B were prepared for phosphorous content determination and ESI-MS assay.

Phosphorus content assay. The total phosphorous content of the oil, the gum and the material A and B was determined, and pretreatment of oil samples was performed according to Yang et al. [11]. Phosphorus analysis was carried out as follows: 100 mg MgO was weighed into a porcelain dish and heated with gas burner. 0.5–2 g of oil were added and ignited with a gas burner to be 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].

Analysis of Phospholipids in the Gums by Electrospray Ionization-Mass Spectrometry (ESI-MS)

Samples of 0.0500 g A and B were taken and placed in two microcentrifuge tubes, and 1.5 ml methanol was added and mixed by swirling for 2 min. After phase separation by centrifugation at 7,000 rpm for 1 min, the top layer was removed and preserved for the ESI-MS assay.

ESI analysis were carried out on a Micromass Quattro II electrospray ionization quadrupole mass spectrometry (State Key Laboratory of Organic Geochemistry, Guangzhou Institute of Geochemistry, Chinese Academy of Sciences) and operated in the positive ion mode. Data were acquired and analyzed by MassLynx NT software. In all cases, analyses were performed at a flow rate of 5 µl/min provided by a Harvard Apparatus model 55–2111 syringe pump and a source temperature of 230 °C. Typical values for capillary and cone voltages were 4 kV and 120 V, respectively. Mass resolution was 8000 (Full Width at Half Maximum, FWHM). The MS data were collected under a full-scan mode in the range of 200–500 m/z at a rate of five MS spectra per time point.

Results and Discussion

Plant trials for enzymatic degumming. Based on the research results in our lab [10, 11], an enzymatic degumming trial of soybean oil was carried out at the 400 tons/day line (Fig. 1).The phosphorus content in the first stage of the reactor decreased from 121.5 mg/kg (crude oil) to 61.2 mg/kg due to coagulation and precipitation of part of the phosphatides by the addition of citric acid. pH played an important role in the enzymatic degumming performance because of different enzyme activity at different pHs (Fig. 2). When the optimal pH for the degumming process was in the range of 4.8–5.1, less than 10 mg/kg of phosphorous content could easily be obtained (Fig. 2). The gum and oil was easily separated after centrifugation, and only 0.6% oil was lost in the gum in comparison with the acid degumming process from which about 15% oil was lost from the gum. The phosphorus content of the oils after bleaching was tested and the mean level was about 3 mg/kg. This was regarded as good enough for the physical refining process. However, the bleaching earth consumption was little higher than the chemical refining process and higher clay consumption resulted in higher oil loss in the clay. Since phospholipids degrade at high temperature, the phosphorous content of the oil, after deodorization, was about 1–2 mg/kg.

During the enzymatic degumming process, interesting phenomena were observed in the gums. Gums were pale-yellow and had good liquidity, while gums from acid degumming was reddish black and viscous. The oil loss was highly reduced.

Phospholipids Composition in the Gums

Clausen [9] had used different enzymes to remove the phospholipids in the rapeseed oil, and the content of lysophospholipids (lyso-PC) and phospholipids was monitored over time by HPLC. In our study, we wanted to know if there were other products besides lyso-PC from the hydrolysis of soybean phospholipids and therefore ESI-MS was used to qualitatively analyze the products. Soybean lecithin usually includes 20% phosphatidylcholine (PC),

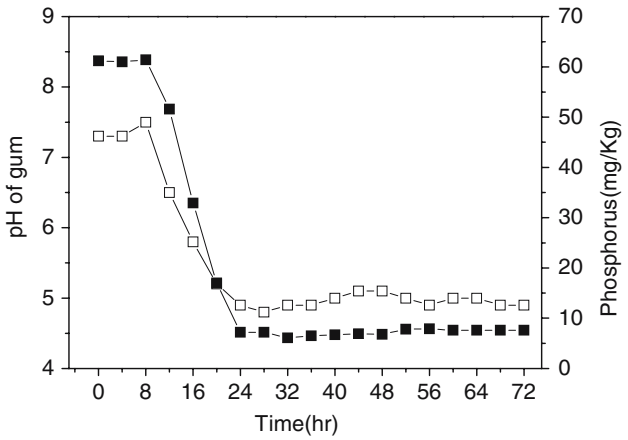

Fig. 2 Results from industrial trial for soybean oil degumming, processing parameters were as follows: flow rate of oil, 17 tons/h; flow rate of 45 citric acid, 20 L/h; flow rate of 10% enzyme solution, 8.8 L/h. The total water content was 2–3 % of oil. The reaction time was 30 min and 6 h in the citric retention tank and reactor, respectively. *open squares* pH of gum, *filled squares* resident phosphorous content in the oil

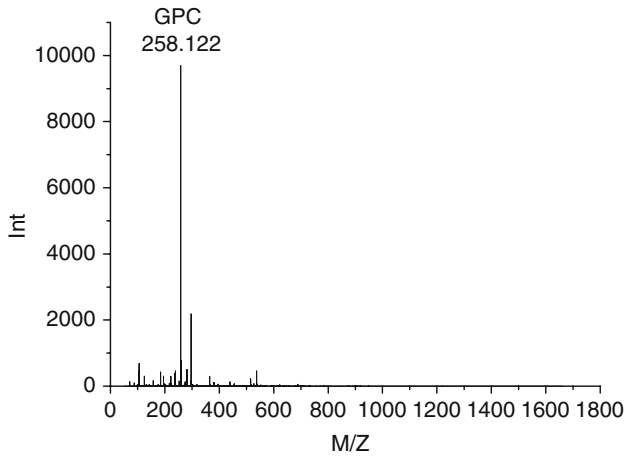

Fig. 3 Positive-ion electrospray mass spectra of material A

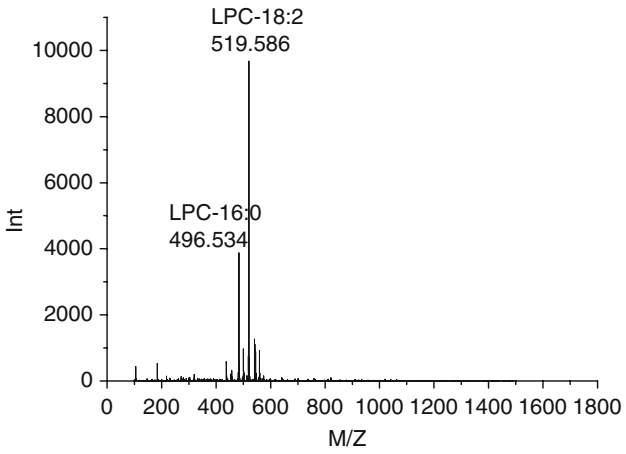

Fig. 4 Positive-ion electrospray mass spectra of material B. The numbers given behind characterize the fatty acid part of LPC (total carbon number: total number of double bonds, such as 18:2)

15% phosphatidylethanolamines (PE), 20% phosphatidylinositol (PI), 5% phosphatidic acid (PA) and other phosphatides [13]. PC, characterized by the presence of a quaternary nitrogen atom whose positive charge is neutralized by the negative charge of the phosphate group, is always ionized and is independent of the solution pH. The quaternary nitrogen atom readily forms an abundant $[M + H]^+$ ion by electrospray ionization because the phosphate anion can be protonated during the electrospray process. PC will produce strong intensities in the positive ion mode, and these often overshadow the lower response of PE. The remaining phospholipids class, PG, PS and PI, are best detected in the negative ion mode, owing to the acidic nature of head groups.

The composition of PC and its hydrolysis product (material A and B; Figs. 3, 4) in the gums was investigated by ESI-MS in the positive ion mode. The molecular weight of compounds in the material A was mainly between 200 and 400 (Fig. 3), and the ion is found at *m/z* 258.122, corresponding to the $[M + H]^+$ ion of glycerophosphatidylcholine (GPC) which was formed by enzymatic hydrolysis of two fatty acyl groups of phosphatidylcholine. The ions spectrum of material B (Fig. 4) was mostly between 450 and 600, which conformed to the m/z extent of lysophosphatidylcholine (LPC). The ion peaks of *m/z* 496.534 and 519.586 corresponded to the $[M + H]^+$ ion of LPC-16:0 and LPC-18:2 respectively. From the above ESI-MS analysis, it could be seen that both GPC and LPC were produced in the enzymatic process and therefore it could be deduced that the phospholipids were hydrolyzed into either glycerophospholipids or lysophospholipids.

The phosphorous content of the gums, material A, and B was analyzed and the results were shown as Table 1. Lysophospholipids existed in the material B, and glycerophospholipids was in the material A. From Table 1, it can be seen that 54.3% of total phospholipids of gums were lysophospholipids, and 45.7% were converted into glycerophospholipids. The emulsifying capacity of lysophospholipids is higher than phospholipids for o/w emulsions, while glycerophospholipids are more hydrophilic and this may explain the reason why the oil phase and gum phase can be separated easily in the enzymatic process by the enzyme, and therefore a lower oil loss was realized. Successful enzymatic degumming seems to be related to the production of glycerophospholipids. This is in accordance with the findings of Clausen [9] when applying phospholipase A_2 whereas he did not document conversion of lyso-phospholipids when degumming with a phospholipase A_1.

Table 1 Phosphorous content of gum water and material A and B in 100 mL gum water

Sample	Total phosphorous content (mg)	Equivalent intact phospholipid content[a] (mg)	Proportion of gum water phospholipid[b] (%)
The gum water	455	10,465	–
Material A	207.93	4782.39	45.7
Material B	247.07	5682.61	54.3

[a] Total phospholipids can be calculated from phosphorus content by multiplication with 23

[b] Conversion ratio of total phospholipids means that 54.3% of total phospholipids were hydrolyzed to be lyso-PC, and 45.7% of total phospholipids were hydrolyzed to be GPC

For a degumming process to be successful, the selection of enzyme and its catalyzed mechanism is very important. In the degumming process by microbial phospholipase A1 from *Thermomyces lanuginosus/Fusarium oxysporum*, the reaction mechanism was not what had been previously reported for phospholipase A_1 (from *Fusarium oxysporum*) with only lysophospholipid production [9, 14] in the lab experiments. Instead it should be: (1) the fatty acid at the sn-1 position of phospholipids is hydrolyzed by phospholipase A_1; (2) spontaneous acyl migration takes place with the fatty acids at the sn-2 position moving to the sn-1 position; (3) phospholipids are hydrolyzed to produce lysophospholipids and glycerophospholipids.

In the enzymatic degumming process, different phospholipids such as PC, PE, PI, PA, lyso -PC, GPC, lyso-PE, GPE, lyso-PI, GPI, lyso-PA, and GPA are produced. Furthermore these phospholipids are different from different oil sources (e.g. rapeseed and soybean oils). A full composition of the gums will be reported in the near future.

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