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The effect of ultrasound on enzymatic degumming process of rapeseed oil by the use of phospholipase A_1 ☆

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ABSTRACT

Comparative studies of enzymatic degumming process of rapeseed oil were carried out in mechanical-stirring and ultrasonic-assisted mechanical-stirring systems. The influences of enzyme dosage (10–50 mg/kg), pH (4.5–6), temperature (45–65 °C), water amount (1–3%), ultrasonic power (0.06–0.09 W/cm^3) and reaction time were investigated subsequently. A suitable ultrasonic power of 0.07 W/cm^3 was determined to guarantee satisfactory degumming efficiency and enzyme activity. Compared to the mechanical-stirring system, optimum temperature of phospholipase A (PLA) in the ultrasonic-assisted mechanical-stirring system was about 5 °C higher, while the effects of pH on both of the two systems were quite similar. Less time and water were used in the ultrasonic-assisted mechanical-stirring system for enzymatic degumming. The study on the quality changes of degummed oils showed that ultrasound could accelerate the oxidation of edible oils due to the effect of cavitation, thus more attention should be paid on the oxidative stability in the further application.

1. Introduction

Rapeseed is one of the most important oilseed crops and a major source of edible vegetable oil in China. The planting area of the rapeseed is about 6.7 million hectares, and the total yield is about 12 million tons, accounting for 20% of the world's supply [1]. To obtain edible vegetable oils from oil-bearing seeds such as soybean, rapeseed, peanut or sunflower, various refining operations are required. Degumming is the first step in the refining process of vegetable oils, which removes phospholipids and mucilaginous gums. The presence of substantial amounts of phospholipids can cause oil discoloration and serve as a precursor of off-flavors. Therefore, the removal of nearly all of the phospholipids is essential for the production of high-quality finished oil [2]. Traditional degumming processes, including water degumming, super-degumming, total degumming, acid treatment, etc., cannot guarantee the achievement of low phosphorus contents (<10 mg/kg) required for physical refining, and are not always optimally suited for all oil qualities [3]. Besides, some beneficial minor components such as tocopherols and sterols would be reduced during the alkali refining process followed by the traditional degumming processes. The yield loss, the apparatus requirement and the energy expenditure of these processes are also great [4].

Enzymatic degumming is probably the best process available today for reducing the phosphorus content of vegetable oils below 10 mg/kg. It was first developed in the 1990s in initial industrial plant trials by the German Lurgi Company, as the "EnzyMax process". In this process, enzymes changed non-hydratable phospholipids (NHP) into a hydratable form, which were then removed by centrifugation [5]. In recent years, enzymatic degumming processes have been explored by other researchers with different sources of phospholipases to obtain a low phosphorous content (<10 mg/kg) under suitable conditions [6,7]. Until now, most of the reported studies are focused on the degumming efficiency of phospholipase A (PLA). In the case of Lecitase Ultra, the phosphorous contents of rice bran oil and soybean oil were enzymatically degummed to less than 5 mg/kg and 6 mg/kg after 6.5 h and 5 h, respectively [8,9]. The use of PLA could effectively reduce the phosphorous content of vegetable oils to less than 10 mg/kg to make it suitable for physical refining, and decrease the amounts of acid and alkali used and wastewater generated during the refining process, resulting in an enhancement in product yield and a reduction in operating cost [10]. However, it is quite time-consuming (4–6.5 h) for the treatment of PLA to reduce the phosphorous to less than 10 mg/kg.

Phospholipase-catalyzed reaction takes place at the interface between the aqueous phase containing the enzyme and the oil phase, which means that the rate of this reaction increases when the interfacial area is increased [5]. Mukataka et al. [11] firstly

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proposed a kinetic model for enzyme reactions by taking into account the interfacial area. Ultrasound irradiation, a mechanical rather than an electromagnetic wave, is an alternative method to increase such interfacial area in enzymatic reactions [12]. Though cavitation is unlikely to occur in oil due to the low vapor pressure, ultrasound can cause cavitation in water/oil emulsions. When cavitation bubbles collapse near the phase boundary of two immiscible liquids, the resultant shock wave can provide a very efficient stirring/mixing of the layers, resulting in an enhancement of heterogeneous reactions and forming transient reactive species. Thus, ultrasound can be a useful tool in enzymatic reactions [13]. Though some studies have shown that appropriate treatment of ultrasound can enhance the activity and reaction rate of some kinds of enzymes [13–15], a little work has been devoted to the enzymatic degumming process of vegetable oils enhanced by ultrasonic irradiation.

In this paper, we investigated the effect of ultrasound on enzymatic degumming process of rapeseed oil as compared to the conventional mechanical-stirring enzymatic degumming process, and studied the quality changes of degummed oils treated by the two systems.

2. Experimental

2.1. Materials

Crude rapeseed oil was kindly provided by Jin Taiyang oil Ltd. (Nantong, China) with an original phosphorus content of 252.05 ± 0.91 mg/kg. Phospholipase A_1 (Lecitase Ultra) was purchased from A/S Novozymes (Bagsvaerd, Denmark) and the phospholipase activity was assayed and found to be 8670 U/g. All other reagents were of analytical grade and were purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China), and the solutions were prepared in deionized water.

2.2. Equipment

Mechanical stirring experiments were carried out in a thermostatic water bath equipped with an agitation unit (IKA, Germany, model Eurostar 40 digital; 30–2000 r/min). The agitation unit consisted a 4-bladed propeller-type impeller with a diameter of 50 mm (IKA, Germany, model R1342), and the reaction temperature could be maintained within ±0.2 °C of the desired temperature.

Ultrasound-assisted enzymatic degumming reactions were carried out using an ultrasonic water bath (Hechuang, China, model KH 300GDV, temperature accuracy ±1.0 °C) in which a 250 ml conical flask was immersed with an agitation unit (IKA, Germany, model Eurostar 40 digital; 30–2000 r/min) for enzymatic degumming reactions. The agitation unit consisted a 4-bladed propeller-type impeller with a diameter of 50 mm (IKA, Germany, model R1342). The operating frequency of the ultrasonic bath was 40 kHz and the output power could be adjusted from 50% to 100% of the total power (300 W), and the delivered power dissipated in the medium were estimated by the calorimetric method.

2.3. Determination of PLA_1 activity

PLA_1 assay was performed with deoiled soy lecithin (PL) emulsion using the method of Yang et al. [4]. One unit of PLA_1 (U) is the amount of enzyme which releases 1 μmol of titratable free fatty acid (FFA) per minute under the described conditions. Substrate solution: 25% PL and 4% polyvinyl alcohol solution were emulsified at a volume ratio of 1:4. Analysis conditions: 4 ml of PL emulsion, 5 ml of 0.01 M citric acid buffer (pH 5.0), and 1 ml of enzyme solution were mixed and incubated at 37 °C for 10 min. The reaction was terminated with the addition of 95% ethanol (15 ml) after incubation, and the liberated FFA were titrated with 0.05 M NaOH. Blanks were measured with heat-inactivated PLA_1 samples (95 °C, 10 min). All experiments were carried out in triplicate for the calculation of the mean value.

2.4. Enzymatic degumming without ultrasonic-assisted treatment

Enzymatic degumming process of rapeseed oil by the use PLA_1 was modified according to the method of Yang et al. [16]. Crude rapeseed oil (150 g) was placed into a 250 ml conical flask fitted with an agitation unit (seen in Section 2.2). The oil was heated to about 70 °C in a water bath, and 0.2 ml of 45% citric acid was added under high shear rate (2000 r/min) for 1 min. Then the mixture was allowed to condition for 20 min at 70 °C under stirring (500 r/min). Afterwards, the temperature of the oil was decreased to 50 °C. It was followed by the addition of NaOH solution (16%, w/w) to make the mixture at required pH and 3 ml deionized water were added with high shear mixing (2000 r/min). The required quantity of enzyme was then added and the mixture was mixed under high shear rate (2000 r/min) for 1 min. Then, the mixture was conditioned at 50 °C with mechanical stirring at 500 r/min for enzymatic degumming reactions. After a certain time of enzymatic treatment, the oil mixture was heated to 95 °C for 10 min to inactive the enzyme and then quickly transferred to a centrifuge and centrifuged at 10 000 r/min (11 000*g*) for 10 min. The supernatant fluid was collected and dried by the rotary evaporator at 0.09 Mpa, 80 °C for residual phosphorous content and other analysis. All reactions were carried out in triplicate.

2.5. Enzymatic degumming with ultrasonic-assisted treatment

The first several steps of the experiments were carried out as same as the enzymatic degumming reactions without the ultrasonic-assisted treatment (seen in Section 2.4). After the addition of enzyme, the mixture was quickly placed in the ultrasonic reaction tank consisting of a mechanical agitation unit (seen in Section 2.2). The enzymatic degumming reactions were carried out under the mechanical stirring at 500 r/min in combination with the ultrasonic irradiation at a required reaction temperature. After a certain time of enzymatic treatment, the oil mixture was heated to 95 °C for 10 min to inactive the enzyme and then quickly transferred to a centrifuge and centrifuged at 10 000 r/min (11 000*g*) for 10 min. The supernatant fluid was collected and dried by the rotary evaporator at 0.09 Mpa, 80 °C for residual phosphorous content

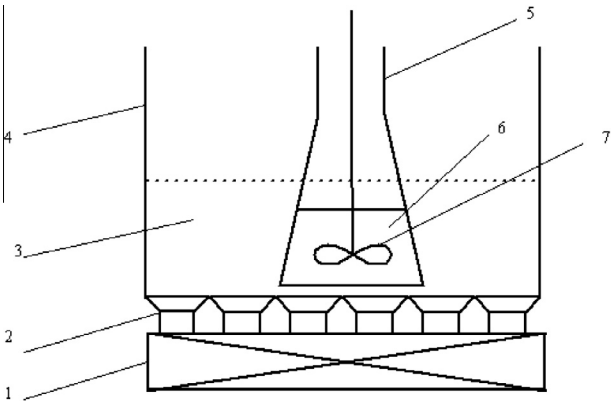

Fig. 1. The schematic diagram of ultrasonic equipment (1-ultrasound producer, 2-ultrasound transducer, 3-water, 4-cleaning tank, 5-reactor, 6-oil mixture, 7-mixer).