Research Article

Effect of Phospholipase A1-Catalyzed Degumming on Oryzanol, Tocopherols, and Tocotrienols of Dewaxed Rice Bran Oil

Sun Xiaoyang,1,2 Tian Shaojun,2 Zhang Lifen,2 and Xie Jianchun1

1Beijing Advanced Innovation Center for Food Nutrition and Human Health, Beijing Technology and Business University (BTBU), Beijing 100048, China
2College of Food Science and Technology, Henan University of Technology, Zhengzhou 450001, China

Correspondence should be addressed to Tian Shaojun; shaojun_tian@haut.edu.cn and Xie Jianchun; xjchun@th.btbu.edu.cn

Received 12 December 2018; Accepted 24 February 2019; Published 27 March 2019

Abstract

The effect of phospholipase A1-catalyzed degumming on the phosphorus content, the retention rate of oryzanol, and total tocopherols and tocotrienols of dewaxed rice bran oil was investigated with comparison to water degumming and citric acid degumming. The fatty acid composition of dewaxed rice bran oil was also studied by gas chromatography. The phosphorus content of dewaxed rice bran oil after phospholipase A1-catalyzed degumming could be decreased from 332.5 mg·kg⁻¹ to 9.3 mg·kg⁻¹ with the citric acid dosage of 0.10%, high shearing rate of 23000 rpm, chelation time of 60 min, NaOH dosage of 1.5 mole equivalent to the amount of citric acid, reaction temperature of 50°C, and total water dosage of 2.5%, while the phosphorus content of dewaxed rice bran oil after water and acid degumming was 120.5 mg·kg⁻¹ and 66.4 mg·kg⁻¹, respectively. The retention rate of oryzanol and total tocopherols and tocotrienols was 97.58% and 96.81% for phospholipase A1-catalyzed degumming, 91.44% and 85.98% for water degumming, and 92.85% and 87.75% for acid degumming. There was no obvious change in fatty acid composition. The results indicated that phospholipase A1-catalyzed degumming was an effective method since it could decrease the phosphorus content to the required level and provide high retention rate of oryzanol and total content of tocopherols and tocotrienols without obvious change of fatty acid composition.

1. Introduction

Rice bran oil has been regarded as nutritious and healthy oil due to its balanced fatty acid profile and has been receiving increasing attention [1]. In addition, it is also rich in bioactive phytochemicals such as oryzanol, tocopherols, tocotrienols, and plant sterols [2–5]. Although the potential production of rice bran oil worldwide is 8 MMT, currently less than 10% of it is processed for edible oil [6]. Rice bran oil obtained from solvent extraction methods is a complex mixture of triacylglycerols, free fatty acids, phospholipids, glycolipid, oryzanol, sterol, tocopherols, and other minor compounds, which makes it difficult to refine [7]. Degumming is the key step in the refining process of crude rice bran oil to remove the phospholipids, which would cause oil discoloration and off-flavors and influence subsequent steps of deacidification, bleaching, and deodorization [8]. If the phosphorus content (P content) after degumming process could be decreased to less than 15 mg·kg⁻¹, preferably less than 10 mg·kg⁻¹, it will benefit the refining process. Hence, the degumming process is essential for rice bran oil with good quality.

Phospholipids in oils are usually present as hydratable and nonhydratable phosphatides. Hydratable phosphatides mainly include phosphatidylcholine and phosphatidylinositol, while nonhydratable phosphatides are primarily composed of phosphatidylethanolamine and calcium and magnesium salts of phosphatic acid, which can be converted into hydratable form under acid conditions [9]. A range of different techniques and processes, including water degumming [10], acid degumming [11], superdegumming [12], total degumming [13], ultrafiltration degumming [6], and enzymatic degumming [14, 15], were developed to remove the phospholipids. Chemical degumming results in high oil losses as well as partial destruction of bioactive
phytochemicals. Water degumming as a physical process can reduce these losses, but this method alone cannot reduce the phosphorus content to the extent required. Enzymatic degumming is probably the best process to reduce the P content of rice bran oil to desirable level as well as retain the desirable bioactive components [16]. In addition, enzymatic degumming also satisfies the increasing demand of environment-friendly technology.

The typical enzymatic degumming was Lurgi’s Enzymax process, which made use of phospholipase to remove the nonhydratable phosphatides (NHPs) [17]. The basic stages of enzymatic degumming include (a) bringing the phospholipids to the water/oil interface by adding citric acid to chelate metals and emulsifying the mixture to provide a large surface; (b) converting phospholipids into lysoform during enzymatic process; and (c) removing the water and phospholipids using centrifugation under low water content. The P content of rapeseed, soybean, and sunflower oils could be reduced to less than 10 mg·kg⁻¹ and even less than 5 mg·kg⁻¹ after enzymatic degumming [18, 19], and this satisfies the requirement for physical refining [20].

Phospholipases, as the critical factor for enzymatic degumming, mainly include phospholipase A₁ (PLA₁), phospholipase A₂, and phospholipase C, which remove the fatty acid from position 1, position 2, and position 3 with respect to glycerol, respectively. The reported PLCs have specificity for phosphatidylcholine and phosphatidylethanolamine, which together represent about 38.2% of the specificity for phosphatidylcholine and phosphatidylethanolamine, respectively. The performance of these phospholipases shows a close correlation with pH and reaction temperature.

So far, there were several studies about the effect of enzymatic degumming on P content, as well as the quantitative and qualitative analysis of phospholipids. However, few researchers have studied the effect of key process parameters of enzymatic degumming on P content, as well as the retention rate of bioactive accompaniments and oil composition. The objectives of this work were to evaluate the effect of key process parameters of PLA₁-catalyzed degumming process on P content and the fatty acid composition of dewaxed rice bran oil and its effect on the retention rate of oryzanol and total content of tocopherols and tocotrienols, compared to water degumming and citric acid degumming.

2. Materials and Methods

2.1. Materials. Dewaxed rice bran oil (moisture, 0.18 ± 0.02%; acid value, 22.72 ± 0.32 mg·kg⁻¹; P content, 332.47 mg·kg⁻¹) was donated by Yihai (Jiamusi) Grain and Oil Industry Co., Ltd., and carefully stored under 5°C in a refrigerator (Haier). Lecitase® Ultra is a protein-engineered carboxylic ester hydrolase (EC 3.1.1.3) extracted from Thermomyces lanuginosus/ Fusarium oxysporum and produced by submerged fermentation of a genetically modified Aspergillus oryzae microorganism (activity, 10 KLU·g⁻¹; temperature, 40–60°C; pH, 5–9) and was procured as free sample from Novozymes (China). Standards of α-tocopherol (purity ≥ 96.0%), β-tocopherol (purity ≥ 96.0%), γ-tocopherol (purity ≥ 96.0%), δ-tocopherol (purity ≥ 99.0%), α-tocotrienol (purity ≥ 98.0%), γ-tocotrienol (purity ≥ 99.0%), and δ-tocotrienol (purity ≥ 99.0%) were purchased from Sigma-Aldrich (St. Louis, USA) and used without any further purification. Diethylene oxide, n-hexane, and isopropanol were of HPLC grade and purchased from VBS Biologic Inc. (New York, USA). All other reagents and solvents were of analytical grade and purchased from Sigma-Aldrich Co. Ltd.

2.2. Dewaxed Rice Bran Oil Degumming Process. For PLA₁-catalyzed degumming process, dewaxed rice bran oil (100 g) was placed in a Duran bottle (250 mL), fitted with mechanical stirrer and thermostat. The oil was heated to minimum 70°C using water bath, and then 222 μL of citric acid solution (45 g/100 mL) was added. After homogenization for 30 sec at 23,000 rpm, the mixture was incubated for 30 min at 70°C, under magnetic stirring (200 rpm). Then, the temperature was decreased to set temperature. 100 mg·kg⁻¹ of sodium hydroxide was added as solution (16 g/100 mL) to achieve soap content of no less than 50 mg·kg⁻¹ and a pH of approximately 5.0 in the water extraction phase. Afterwards, 2.5 mL/100 g of water was added. With the further addition of enzyme of 100 mg·kg⁻¹, the system was mixed under high shear rate (23,000 rpm) for 30 sec to provide a large surface area through emulsification. Then, the temperature was maintained at the set temperature for 2 hours during the enzymatic process. The oil was stirred with magnetic mixer (approximately 200 rpm) during the whole process. The sample was centrifuged at 5000 rpm for 10 min, and the oil at top layer was collected for analysis of P content, oryzanol content, and total content of tocopherols and tocotrienols.

For water degumming process, dewaxed rice bran oil (100 g) was placed in a Duran bottle (250 mL), fitted with mechanical stirrer and thermostat. The oil was heated to minimum 80°C with water bath, followed by the addition of deionized water (2.5%, w/w). The oil was stirred using the magnetic mixer (approximately 200 rpm) for 50 min during the whole process. The stirring rate was slowed down for 10 min for good flocculation. The sample was centrifuged at 5000 rpm for 10 min, and the oil at top layer was collected for analysis of P content, oryzanol content, and total content of tocopherols and tocotrienols.

For citric acid degumming process, the same process was used as the PLA₁-catalyzed degumming process for the chelation step. Then, the oil was heated to minimum 80°C using water bath, and the oil was stirred with magnetic mixer (approximately 200 rpm) for 20 min. The stirring rate was slowed down for 10 min for good flocculation. The sample was centrifuged at 5000 rpm for 10 min, and the oil at top layer was collected for analysis of P content, oryzanol content, and total content of tocopherols and tocotrienols.
2.3. Analysis of P Content. 100 mg of ZnO was weighed in a porcelain dish and heated on a gas burner. A mass of 1 g of oil was added ignited with a gas burner to become a black and hard mass, and then it was heated at 850°C for 2 hours until it turned into white ash. The P content of the ash was determined according to the AOCS method Ca 12-55 [24]. All experiments were carried out in triplicate for the calculation of the mean value.

2.4. Analysis of Nonhydratable Phosphatide. A 100 g of oil was weighed and heated to 65°C. Once the oil reached 65°C, 10 mL of Mili-Q water was added, and the stirring was to continue for 20 min. The stirring rate was slowed down for 10 min for good flocculation. The sample was centrifuged at 5000 rpm for 10 min, and the oil at top layer was collected for P content analysis.

2.5. Analysis of Fatty Acid Composition by GC. The substrate was initially methylated to fatty acid methyl esters (FAME) according to the AOCS Official Method Ce 2–66 [25]. The final FAMEs were determined using gas chromatography (Agilent 6890N; Agilent Corp, America) equipped with column SGE BPX-70 (30 m × 0.25 mm i.d., 0.25 μm film thickness) and flame ionization detector (FID). The injection and detector temperatures were 250°C and 300°C, respectively. The oven temperature was increased from 170°C to 210°C at 2°C min⁻¹ and held for 5 min. High purity nitrogen was used as the carrier gas with a flow rate of 1.2 mL min⁻¹. The injection volume was 1 μL with a split ratio of 20:1. FAMEs were identified by comparing their retention times with external standards, and the content of each fatty acid was calculated as a percentage based on peak area.

2.6. Determination of the Content of Oryzanol. The content of oryzanol in dewaxed rice bran oil before and after enzymatic degumming catalyzed by Lecitase Ultra was determined according to the method of Gopala Krishna et al. with minor modification [26]. 10 mg of oil was dissolved in hexane, made up to 10 mL, and mixed well. The OD was read in a 1 cm cell at 314 nm in a Persee TU-1900 double-beam UV-visible recording spectrophotometer (solutions having OD more than 1.2 were further diluted before reading OD). The content of oryzanol was calculated using the formula:

\[
\text{oryzanol (g/100g)} = \frac{\text{OD of hexane solution}}{\text{weight (g) of oil} \times 10^4} \times 100/358.9
\]

where 358.9 is the specific extinction coefficient of oryzanol.

Oryzanol content was calculated by using the following formula: \([A/W] \times (100/358.9)\), where \(A\) is the absorbance of the sample, \(W\) is the weight of the sample in gram/100 mL, and 358.9 is \(E_{1cm}\) 1% for oryzanol.

2.7. Determination of Total Content of Tocopherols and Tocotrienols. The total content of tocopherols and tocotrienols of the dewaxed rice bran oil was determined according to Li et al. with minor modification [27]. A total of 0.50 g of the oil samples was added into a 10 mL volumetric flask, and hexane was added to make up 10 mL and mixed well. The solution was filtered through a 0.45 μm membrane filter before HPLC analysis.

Total content of tocopherols and tocotrienols was analyzed by a normal-phase high-performance liquid chromatograph (NP-HPLC) (Waters 2695) equipped with a fluorescence detector (Waters 2475) set at an excitation wavelength of 290 nm and an emission wavelength of 330 nm, and the detailed conditions are as follows: the injection volume for each of samples was 20 μL; the column was Sunfire Prep Silica (4.6 × 250 mm, 5 μm, Waters); the mobile phase consisting of hexane/diethylene oxide (96.15/3.85 v/v), degassed for 40 min by an ultrasonic apparatus, was eluted at a constant flow rate of 1.0 mL min⁻¹. Retention times of the standards of tocopherols and tocotrienols were used for identification. Quantification of the tocopherols and tocotrienols was done using an external calibration curve, and the total content of tocopherols and tocotrienols was reported in mg kg⁻¹.

2.8. Retention Rate. The retention rate of oryzanol and total tocopherol and tocotrienol was defined as follows:

\[
\text{retention rate} = \frac{C_T \times Y}{C_0},
\]

where \(C_T\) is the content of oryzanol or the content of total tocopherol and tocotrienol of rice bran oil after degumming, \(Y\) is the yield of rice bran oil after degumming, and \(C_0\) is the initial content of oryzanol or the content of total tocopherol and tocotrienol of rice bran oil.

2.9. Statistical Analysis. All experiments were conducted independently in triplicate, and the results were expressed as mean ± standard deviation. The significance of the differences was determined using one-way analysis of variance (ANOVA), accompanied by Duncan’s post hoc multiple comparison test with a significance level of 0.05, using SPSS 16.0 software (International Business Machines Co. Armonk, NY, USA). Differences were considered significant when the \(P\) value was <0.05.

3. Results and Discussion

3.1. Effect of Citric Acid Dosage and Mixing Conditions on NHP Content. The P content of dewaxed rice bran oil was 332.5 ± 1.9 mg kg⁻¹, of which 120.5 ± 2.3 mg kg⁻¹ was for the nonhydratable phosphatides. Citric acid was used in the pretreatment step for facilitating hydration of the phosphatides and to buffer the aqueous phase, and moreover, high shear mixing was chosen for high reaction efficiency. The P content of dewaxed rice bran oil was difficult to be reduced to the desired level for physical refining because of the high content of NHPs, which is needed to be converted into hydratable form as far as possible [9]. Both the dosing of citric acid and high efficiency mixing condition could bring the phospholipids to the water/oil interface and provide a large surface, and it would benefit the enzyme to convert...
phospholipids into the lysoform and remove the water and phospholipids using centrifugation under low water content [15, 19]. As shown in Figure 1, the pretreatment with the dosage of citric acid for 0.065%, 0.10%, and 0.13% reduced NHP content to 76.9 ± 0.4 mg·kg⁻¹, 66.4 ± 1.1 mg·kg⁻¹, and 64.7 ± 0.7 mg·kg⁻¹, respectively, after 60 min of incubation with premixing rate of 23000 rpm for 30 sec. To reduce the NHP content to a lower value, the dosage of citric acid was no less than 0.10%.

These results were close with the previous studies using the acid degumming. The P content of dewaxed rice bran oil was reduced from 350 mg·kg⁻¹ to 86 mg·kg⁻¹ after acid degumming, and this was probably because of the increased phosphate hydration [28]. Hence, the P content was expected to be reduced to less than 15 mg·kg⁻¹ for physical refining with the addition of phospholipase A₁.

3.2. Effect of Processing Parameters of PLA₁-Catalyzed Degumming on P Content. Enzymatic degumming with Lecitase® Ultra was a combination of a mild acid treatment to convert Ca and Mg salts to a form that the enzyme could attack with an enzyme hydrolysis to make all the gums hydrophilic. More and more oil is released from the gums because they were hydrophilic resulting from the partial hydrolysis of phosphatides, and the key point was to ensure enzyme hydrolyze phosphatides efficiently. There were some typical inhibitors including extreme temperature, extreme pH, metals, and chelators (EDTA and phosphates) for the performance of enzymes. The Lecitase® Ultra, used for the degumming of dewaxed rice bran oil, had the proper range of pH and reaction temperature and would have the high reaction activity if the reaction conditions could be adjusted and maintained according to its proper pH and temperature [29].

As to ensure the optimum pH of the water extraction phase for Lecitase® Ultra, the NaOH solution of approximately 16 g/100 mL was added, and the solution was further diluted with water to reach the selected total water dosage. The effect of NaOH dosage on P content was investigated under the following conditions: citric acid dosage of 0.10%, degumming temperature of 50°C, total water dosage of 2.5%, degumming time of 4 hours, and NaOH dosage of 1.0, 1.5, 2.0, 2.5, and 3.0 mole equivalent (equiv) to the amount of citric acid, respectively. As shown in Figure 2(a), the P content of dewaxed rice bran oil after PLA₁-catalyzed degumming decreased significantly with increasing NaOH dosage and reached a minimum at a NaOH dosage of 2.0 equiv. More NaOH would result in more soap, which might damage the stability of emulsion interface. Phospholipid may remain in the oil, which may be dragged to the heavy phase if the interface was not well defined, and this probably would affect the performance of Lecitase® Ultra. The residual P content of dewaxed rice bran oil decreased with the increasing reaction time. As to reduce the P content to less than 10 mg·kg⁻¹, the reaction time should not be less than 4 hours, and this was similar with the report from Jahani et al. [28].

The temperature of water degumming was approximately 80°C, while the enzyme was easily inactivated under this temperature. In addition, low temperatures were not suitable for enzymatic reactions [30]. The effect of reaction temperature on P content was investigated under the following conditions: citric acid dosage of 0.10%; NaOH dosage of 2.0 eqv; total water dosage of 2.5%; degumming time of 4 hours; reaction temperature of 40°C, 45°C, 50°C, 55°C, and 60°C, respectively. As shown in Figure 2(b), the P content of dewaxed rice bran oil after PLA₁-catalyzed degumming decreased significantly with increasing reaction temperature and reached a minimum at a reaction temperature of 50°C. High reaction temperature would damage the enzyme activity, and as a result of that, the P content had no obvious change from 3 hours to 4 hours with reaction temperature of 60°C.

The effect of water dosage on P content was investigated under the following conditions: citric acid dosage of 0.10%; NaOH dosage of 2.0 eqv; degumming time of 4 hours; reaction temperature of 50°C; and total water dosage of 1.5%, 2.0%, 2.5%, 3.0%, and 3.5%, respectively. As shown in Figure 2(c), the P content of dewaxed rice bran oil after PLA₁-catalyzed degumming decreased significantly with increasing total water dosage and reached a minimum at a total water dosage of 2.5%. The stable multilayer liposome structure could be formed by adding proper amount (2.5%) of water. However, low water content resulted in incomplete hydration and poor flocculation, while high water content would promote to form water/oil or oil/water emulsification, which made it difficult for the separation [10].

![Figure 1: Effect of citric acid dosage and mixing conditions on NHP content. Note. SA: dewaxed rice bran oil without addition of citric acid; SB: dewaxed rice bran oil with 0.065% citric acid added; SC: dewaxed rice bran oil with 0.10% citric acid added; SD: dewaxed rice bran oil with 0.13% citric acid added; IA: high shear mixer of 10000 rpm for 30 sec after 30 min of incubation; IB: high shear mixer of 10000 rpm for 30 sec after 60 min of incubation; IC: high shear mixer of 20000 rpm for 30 sec after 60 min of incubation; ID: high shear mixer of 23000 rpm for 30 sec after 60 min of incubation. Uppercase superscripts represent significant differences between groups; lowercase superscripts represent significant differences within a group (P < 0.05).](attachment:image.png)
3.3. Comparison of Water, Acid, and PLA$_1$-Catalyzed Degumming on P Content. The adequate reduction of phosphorus content resulted from a synergistic effect of enzyme and citric acid. To obtain an efficient enzyme reaction, the formation of emulsion was crucial, and it could be obtained by applying high shear mixer prior to the enzyme reactor. Mechanical stirring was maintained throughout the reaction, although the oil/water mixture may be subjected to more treatments with high shear mixer to keep the mixture properly emulsified. The oil/water mixture was heated to 80 °C and separated into two phase (a sludge-free oil phase and an oil-free sludge phase) after the reaction ended.

As shown in Table 1, the P content of dewaxed rice bran oil could be reduced from 332.5 ± 1.9 mg·kg$^{-1}$ g to 9.3 ± 0.2 mg·kg$^{-1}$ after PLA$_1$-catalyzed degumming using Lecitase Ultra under the optimum conditions, namely, citric acid dosage of 0.10%, high shearing rate of 23000 rpm, chelation time of 60 min, NaOH dosage of 1.5 eqv, reaction temperature of 50 °C, and total water dosage of 2.5%, whereas the P content after water and acid degumming were reduced up to 120.5 ± 2.3 mg·kg$^{-1}$ and 66.4 ± 1.1 mg·kg$^{-1}$, respectively.

For the water degumming, the residual P content of dewaxed rice bran oil could not be reduced to less than 50 mg·kg$^{-1}$ at optimum condition [10]. The lecithin, obtained by drying the gums resulted from rice bran oil water degumming, was composed of 20.4% PC, 17.8% PE, and 5.8% PI [22], and only hydratable phosphatides could be separated by water addition. The P content of rice bran oil

![Figure 2: Effect of processing parameters of PLA$_1$-catalyzed degumming on P content. Note: uppercase superscripts represent significant differences between groups; lowercase superscripts represent significant differences within a group (P < 0.05).](image)
after water degumming could not satisfy the requirement for physical refining (less than 15 mg·kg⁻¹). Acid degumming was applied based on the addition of phosphoric acid or citric acid to convert the nonhydratable phosphatides into hydratable ones [31], but the residual P content more than 50 mg·kg⁻¹ also could not fit the physical refining. Furthermore, the condition of PLA₁-catalyzed degumming (temperature of 50 °C) was more moderate in comparison to the condition of water degumming (80 °C) and acid degumming (70 °C). Therefore, PLA₁-catalyzed degumming of dewaxed rice bran oil was considered an efficient method for dewaxed rice bran oil compared to water and acid degumming.

3.4. Comparison of Water, Citric Acid, and PLA₁-Catalyzed Degumming on Fatty Acid Composition, Oryzanol Content, and Total Tocopherol/Tocotrienol Content. The nutritional properties of oryzanols, tocopherols, and tocotrienols have been reported in a series of scientific studies. Oryzanol could reduce the total plasma cholesterol, increase HDL cholesterol levels, and inhibit platelet aggregation [32]. Tocopherols could prevent coronary heart disease and cataract formation, lower the levels of plasma triacylglycerols and cholesterol, delay aging, cancer, and arthritis, and reduce oxidative stress. Tocotrienols could decrease serum and LDL-cholesterol levels and have significant activity against tumours [33]. According to the above information, the target of rice bran oil processing, no matter of chemical or physical refining, should be not only to remove the impurities, but also to obtain good oil with no obvious change of fatty acid composition and high retention rate of oryzanol and total tocopherols and tocotrienols.

As shown in Table 2, the content of C16:0, C18:0, and C18:3 of dewaxed rice bran oil after different degumming methods had no obvious change (P < 0.05). Meanwhile, the content of C18:1 and C18:2 of dewaxed rice bran oil after water, citric acid, and enzymatic degumming were still higher than other fatty acids [34], and there were no significant differences between the three degumming methods (P < 0.05).

Table 1: Effect of degumming methods on P content.

| Degumming process       | Water degumming        | Acid degumming        | PLA₁-catalyzed degumming |
|-------------------------|------------------------|-----------------------|--------------------------|
| P content (mg·kg⁻¹)     | 120.5 ± 2.3ᵃ           | 66.4 ± 1.1ᵇ           | 9.3 ± 0.2ᶜ              |

Note: lowercase superscripts represent significant differences between groups (P < 0.05).

Table 2: Effect of degumming methods on fatty acid composition.

| Fatty acid | Dewaxed rice bran oil | Water degumming | Acid degumming | PLA₁-catalyzed degumming |
|------------|-----------------------|-----------------|----------------|--------------------------|
| C14:0      | 0.25 ± 0.03ᵇ          | 0.40 ± 0.01ᵃ    | 0.20 ± 0.00ᶜ  | 0.00 ± 0.00ᵈ             |
| C16:0      | 17.36 ± 0.18⁴         | 17.09 ± 0.06ᵇ   | 17.15 ± 0.11⁴ | 17.25 ± 0.10⁴            |
| C18:0      | 1.47 ± 0.10⁰          | 1.44 ± 0.03ᵃᵇ   | 1.32 ± 0.02ᵇ  | 1.44 ± 0.02ᵃᵇ            |
| C18:1      | 40.42 ± 0.20⁰         | 40.33 ± 0.07ᵇ   | 40.84 ± 0.11ᵇ | 40.66 ± 0.22ᵃᵇ           |
| C18:2      | 37.95 ± 0.31⁴         | 38.01 ± 0.10ᵇ   | 38.35 ± 0.11ᵇ | 38.41 ± 0.10ᵇ            |
| C18:3      | 2.26 ± 0.10⁰          | 2.28 ± 0.06ᵇ    | 1.46 ± 0.08ᵇ  | 2.24 ± 0.04ᵃ              |
| C20:0      | 0.29 ± 0.06ᵇ          | 0.45 ± 0.05ᵃᵇ   | 0.69 ± 0.06ᵇ  | 0.00 ± 0.00ᵈ              |

Note: different smallcase letters in the same row represent a significant difference at P < 0.05.
As shown in Figure 3, the retention rate of oryzanol of dewaxed rice bran oil after water, citric acid, and PLA1-catalyzed degumming was 91.44% ± 0.21%, 92.85% ± 0.32%, and 97.58% ± 0.72%, respectively. The retention rate of oryzanol of dewaxed rice bran oil after PLA1-catalyzed degumming was significantly higher than that after both of water and citric acid degumming (P < 0.05). However, little of oryzanol was lost even for PLA1-catalyzed degumming, because the oryzanol reacts with NaOH to form oryzanol sodium salt with improved hydrophilicity, and it was easy to precipitate from oil because of the soap absorption [35].

As shown in Figure 4, the retention rate of total tocopherols and tocotrienols of dewaxed rice bran oil after water, citric acid, and PLA1-catalyzed degumming was 85.98% ± 0.88%, 87.75% ± 1.29%, and 96.81% ± 0.68%, respectively. The retention rate of total tocopherols and tocotrienols of dewaxed rice bran oil after PLA1-catalyzed degumming was significantly higher than that after both water and citric acid degumming (P < 0.05).

### 4. Conclusions

The phosphorus content of dewaxed rice bran oil after PLA1-catalyzed degumming could be decreased from 332.5 mg·kg⁻¹ to 9.3 mg·kg⁻¹ with the citric acid dosage of 0.10%, high shearing rate of 23000 rpm, chelation time of 60 min, NaOH dosage of 1.5 mole equivalent to the amount of citric acid, reaction temperature of 50°C, and total water dosage of 2.5%. However, the phosphorus content of dewaxed rice bran oil after water and acid degumming was 120.5 mg·kg⁻¹ and 66.4 mg·kg⁻¹, respectively. The retention rate of oryzanol and total tocopherols and tocotrienols were 97.58% and 96.81% for PLA1-catalyzed degumming, 91.44% and 85.98% for water degumming, and 92.85% and 87.75% for acid degumming. There was no obvious change for fatty acid composition of dewaxed rice bran oil after water, citric acid, and PLA1-catalyzed degumming. The results indicated that PLA1-catalyzed degumming was an effective method since it could decrease the phosphorus content to the required level and provide high retention rate of oryzanol and total content of tocopherols and tocotrienols without obvious change of fatty acid composition.

### Data Availability

All data supporting the findings of this paper are shown in the tables and figures within the article.

### Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication.

### Acknowledgments

The authors acknowledge the financial support by the Science and Technology Project of Henan Province (182102110253).

### References

[1] R. Pandey and S. L. Shrivastava, “Comparative evaluation of rice bran oil obtained with two-step microwave assisted extraction and conventional solvent extraction,” *Journal of Food Engineering*, vol. 218, pp. 106–114, 2018.

[2] C. Aguilar-Garcia, G. Gavino, M. Baragaño-Mosqueda, P. Hevia, and V. C. Gavino, “Correlation of tocopherol, tocotrienol, γ-oryzanol and total polyphenol content in rice bran with different antioxidant capacity assays,” *Food Chemistry*, vol. 102, no. 4, pp. 1228–1232, 2007.

[3] C. D. S. C. I. Pascual, I. L. Massaretto, F. Kawasaki, R. M. C. Barros, J. A. Noldin, and U. M. L. Marquez, “Effects of parboiling, storage and cooking on the levels of tocopherols, tocotrienols and γ-oryzanol in brown rice (*Oryza sativa* L.),” *Food Research International*, vol. 50, no. 2, pp. 676–681, 2013.

[4] M. Zheng, J. Zhu, F. Huang et al., “Enzymatic deacidification of the rice bran oil and simultaneous preparation of phytosterol esters-enriched functional oil catalyzed by immobilized lipase arrays,” *RSC Advances*, vol. 5, no. 86, pp. 70073–70079, 2015.

[5] X. Wang, J. Lu, H. Liu, Q. Jin, and X. Wang, “Improved deacidification of high-acid rice bran oil by enzymatic esterification with phytosterol,” *Process Biochemistry*, vol. 51, no. 10, pp. 1496–1502, 2016.

[6] S. Zullaikah, C.-C. Lai, S. R. Vali, and Y.-H. Ju, “A two-step acid-catalyzed process for the production of biodiesel from rice bran oil,” *Bioresource Technology*, vol. 96, no. 17, pp. 1889–1896, 2005.

[7] G. Mahua, “Review on recent trends in rice bran oil processing,” *Journal of the American Oil Chemists Society*, vol. 84, no. 4, pp. 315–324, 2007.

[8] L. Mei, L. Wang, Q. Li, J. Yu, and X. Xu, “Comparison of acid degumming and enzymatic degumming process for silybum marianumseed oil,” *Journal of the Science of Food and Agriculture*, vol. 93, no. 11, pp. 2822–2828, 2013.

[9] G. Sengar, P. Kaushal, H. K. Sharma, and M. Kaur, “Degumming of rice bran oil,” *Reviews in Chemical Engineering*, vol. 30, no. 2, pp. 183–198, 2014.

[10] T. N. Indira, J. Hemavathy, S. Khatoon, A. G. Gopala Krishna, and S. Bhattacharya, “Water degumming of rice bran oil: a response surface approach,” *Journal of Food Engineering*, vol. 43, no. 2, pp. 83–90, 2000.

[11] S.-Y. Chen, N. Teerananont, T. Sonthisawat et al., “A cost-effective acid degumming process produces high-quality jatropha oil in tropical monsoon climates,” *European Journal of Lipid Science and Technology*, vol. 117, no. 7, pp. 1079–1087, 2015.

[12] J. C. Segers, “Superdegumming, a new degumming process and its effect on the effluent problems of edible oil refining,” *Fette, Seifen, Anstrichmittel*, vol. 84, no. 1, pp. 543–546, 1982.

[13] B. Gleene newerck and A. J. Dijkstra, “The total degumming process—theory and industrial application in refining and hydrogenation,” *Fett Wissenschaft Technologie/Fat Science Technology*, vol. 94, no. 8, pp. 317–322, 1992.

[14] Z. Li, H. Liu, G. Zhao et al., “Enhancing the performance of a phospholipase A₁ for oil degumming by bio-imprinting and immobilization,” *Journal of Molecular Catalysis B: Enzymatic*, vol. 123, pp. 122–131, 2016.

[15] C. Cerminati, F. Eberhardt, C. E. Elena, S. Peirú, M. E. Castelli, and H. G. Menzella, “Development of a highly efficient oil degumming process using a novel phosphatidylinositol-
specific phospholipase C enzyme,” Applied Microbiology and Biotechnology, vol. 101, no. 11, pp. 4471–4479, 2017.

[16] P. P. Chakrabarti, S. K. Roy, P. R. Karna Narayana et al., “Process for the pre-treatment of vegetable oils for physical refining,” US: US 20040005399A1, 2004.

[17] K. Clausen, “Enzymatic oil-degumming by a novel microbial phospholipase,” European Journal of Lipid Science and Technology, vol. 103, no. 6, pp. 333–340, 2001.

[18] B. Yang, Y.-H. Wang, and J.-G. Yang, “Optimization of enzymatic degumming process for rapeseed oil,” Journal of the American Oil Chemists’ Society, vol. 83, no. 7, pp. 653–658, 2006.

[19] D. L. Lamas, D. T. Constenla, and D. Raab, “Effect of degumming process on physicochemical properties of sunflower oil,” Biocatalysis and Agricultural Biotechnology, vol. 6, pp. 138–143, 2016.

[20] D. Cowan and P. M. Nielsen, “Enzymatic degumming of edible oils and fats,” in Bleaching and Purifying Fats and Oils, AOCS Press, Urbana, IL, USA, 2010.

[21] G. Borrelli and D. Trono, “Recombinant lipases and phospholipases and their use as biocatalysts for industrial applications,” International Journal of Molecular Sciences, vol. 16, no. 9, pp. 20774–20840, 2015.

[22] S. Adhikari and J. Adhikari, “Indian rice bran lecithin,” Journal of the American Oil Chemists’ Society, vol. 63, no. 10, pp. 1367–1369, 1986.

[23] X. Jiang, M. Chang, X. Wang, Q. Jin, and X. Wang, “A comparative study of phospholipase A1 and phospholipase C on soybean oil degumming,” Journal of the American Oil Chemists’ Society, vol. 91, no. 12, pp. 2125–2134, 2014.

[24] AOCS, “Method CA 12-55,” in Official Methods and Recommended Practices of the American Oil Chemists’ Society, AOCS, Champaign, IL, USA, 5th edition, 2003.

[25] AOCS, “Method CE 2-66,” in Official Methods and Recommended Practices of the American Oil Chemists’ Society, AOCS, Champaign, IL, USA, 5th edition, 2003.

[26] A. G. Gopala Krishna, K. H. Hemakumar, and S. Khatoon, “Study on the composition of rice bran oil and its higher free fatty acids value,” Journal of the American Oil Chemists’ Society, vol. 83, no. 2, pp. 117–120, 2006.

[27] J. Li, Y. Bi, S. Sun, and D. Peng, “Simultaneous analysis of tert-butylhydroquinone, tert-butylquinone, butylated hydroxyltoluene, 2-tert-butyl-4-hydroxyanisole, 3-tert-butyl-4-hydroxyanisole, α-tocopherol, γ-tocopherol, and δ-tocopherol in edible oils by normal-phase high performance liquid chromatography,” Food Chemistry, vol. 234, pp. 205–211, 2017.

[28] M. Jahani, M. Alizadeh, M. Pirozifard, and A. Qudsevali, “Optimization of enzymatic degumming process for rice bran oil using response surface methodology,” LWT—Food Science and Technology, vol. 41, no. 10, pp. 1892–1898, 2008.

[29] A. J. Diijkstra, “Enzymatic degumming,” European Journal of Lipid Science and Technology, vol. 112, no. 11, pp. 1178–1189, 2010.

[30] Y. Qu, L. Sun, X. Li et al., “Enzymatic degumming of soybean oil with magnetic immobilized phospholipase A2,” LWT, vol. 73, pp. 290–295, 2016.

[31] N. S. More and P. R. Gogate, “Ultrasound assisted enzymatic degumming of crude soybean oil,” Ultrasonics Sonochemistry, vol. 42, pp. 805–813, 2018.

[32] T. A. Wilson, R. J. Nicolosi, B. Woolfrey, and D. Kritchevsky, “Rice bran oil and oryzanol reduce plasma lipid and lipoprotein cholesterol concentrations and aortic cholesterol ester accumulation to a greater extent than ferulic acid in hypercholesterolemic hamsters,” Journal of Nutritional Biochemistry, vol. 18, no. 2, pp. 105–112, 2007.

[33] R. B. N. Prasad, “Refining of rice bran oil,” Lipid Technology, vol. 12, no. 18, pp. 275–279, 2006.

[34] C. E. C. Rodrigues, M. M. Onoyama, and A. J. A. Meirelles, “Optimization of the rice bran oil deacidification process by liquid-liquid extraction,” Journal of Food Engineering, vol. 73, no. 4, pp. 370–378, 2006.

[35] V. R. Pestana, R. C. Zambiasi, C. R. B. Mendonça, M. H. Bruscatto, M. J. Lerma-García, and G. Ramis-Ramos, “Quality changes and tocopherols and γ-orizanol concentrations in rice bran oil during the refining process,” Journal of the American Oil Chemists’ Society, vol. 85, no. 11, pp. 1013–1019, 2008.
