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Improved deacidification of high-acid rice bran oil by enzymatic esterification with phytosterol

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ABSTRACT

A sustainable method for enzymatic deacidification of high-acid rice bran oil (HRBO) by enzymatic esterification between free fatty acids (FFA) of HRBO and phytosterol is described in this study. The deacidification conditions were optimized to minimize the FFA of HRBO. Under optimal conditions, the FFA content in HRBO reduced from 15.8% to 1.2% (w/w), whereas the phytosterol ester content increased from 0% to 29.3% (w/w). Subsequently, studies on the oxidation stability of deacidified rice bran oil (RBO) indicated that most of the vitamin E was retained in deacidified oil and that the oxidation stability under storage conditions increased after lipase-catalyzed esterification due to the formation of phytosterol ester in the product. Compared to previous methods using glycerol or monoacylglycerol (MAG) as acyl acceptors to reduce the FFA content of HRBO, an improved route was used to deacidify the FFA and produce RBO rich in phytosterol ester with phytosterol as the acyl acceptor. The enzymatic route is greener and more sustainable than traditional deacidification processes.

1. Introduction

Rice bran is composed of 15–25% lipids, which contain about 95% triacylglycerols and 4% unsaponifiable lipids. Unsaponifiable lipids such as tocopherol, tocotrienol, gamma-oryzanol, sterol, and carotenoid have various nutritional benefits; thus, rice bran oil (RBO) is superior to other edible oils [1,2]. Numerous findings have shown that RBO has a lipemia-lowering effect and can reduce the risk of heart disease probably due to the presence of biologically active lipids in RBO [3,4]. The global production of RBO is estimated to be about 7.6 million tons, of which China would account for 1.8 million tons every year. However, only 0.1 million tons of RBO are currently produced in China [5]. Typically, high-acid RBO (HRBO) is used as a raw material for biodiesel production rather than as an edible oil [1,6]. The major limitation of the commercial production of RBO as an edible oil is its free fatty acid content of 30–40%. High FFA content hinders the refining process of RBO, because the loss of HRBO is particularly acute during traditional refining processes. This refining loss includes losses of oil, FFA, and biologically active components. In addition, traditional refining processes also lead to a darker color of RBO [7,8].

The most common method for deacidification of vegetable oils is alkali refining (one kind of chemical refining), but it is generally used for low-FFA crude oils. The refining loss during alkali refining is 2.5–3-fold the FFA content of the oil, and alkali refining also leads to the loss of bioactive components and antioxidants [9]. In addition, this process produces large volumes of wastewater, which pollute the environment. Similarly, the physical refining process also results in refining loss, has stringent requirements in terms of the phosphorus content of the oils, and needs higher energy input than chemical refining [2]. To avoid refining loss, environmental pollution, and high energy input, a more sustainable enzymatic process has been developed for the deacidification of HRBO. The method is popular as it is a much mild, green process, and the beneficial micronutrients will not be destroyed during processing. More importantly, enzymatic deacidification increases oil yield because the FFA is converted to glycerides and other lipids, which can be retained in oils as functional components.

Enzymatic esterification deacidification generally uses glycerol [10–12] and monoacylglycerol (MAG) [13–15] as acyl acceptors to esterify the FFA of high-acid oils. However, the use of glycerol and MAG as acyl acceptors has two drawbacks. First, glycerol and MAG react not only with FFA but also with neutral oil by enzy-

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matic transesterification to produce partial glycerides. This causes the loss of neutral oil [16], leading to a high level of MAG in the final product, which is undesirable. The desirable esterification product is diacylglycerol (DAG), which has been shown to have lipemia-lowering and weight loss effects on humans [17]. Second, subsequent deodorization of oils containing MAG and DAG will lead to the formation of glycidyl esters, which are carcinogenic and genotoxic after they are converted to glycidyls during metabolism [18]. In 1999, Kao Corporation in Japan introduced DAG-rich cooking oil. In 2009, Kao Corporation suspended its DAG oil-based products due to the high content of glycidyl esters detected in these products [19]. Therefore, using glycerol and MAG as acyl acceptors may not be a good choice for the deacidification of HRBO.

In this study, we provided an alternative to neutralize FFA in oils by enzymatic esterification using phytosterol as an acyl acceptor. Since 1999, phytosterol and phytosterol ester have been approved by the United States, Japan, European Union, and China for use in edible healthy materials. Phytosterol and its ester are unsaponifiable components and have many beneficial physiological effects including cholesterol-lowering [20], anti-inflammatory, antiatherogenic, and anticancer effects [21,22]. In the current study, phytosterol was used as an acyl acceptor for the deacidification of HRBO to produce phytosterol ester-rich RBO. Our objectives were to reduce FFA content and increase the functionality of RBO by incorporating phytosterol ester into RBO.

2. Materials and methods

2.1. Materials

High-acid rice bran oil (HRBO, 15.8 wt% FFA) and commercial rice bran oil (CRBO, FFA < 0.1 wt%) were provided by Delekang Food Co., Ltd (Zhejiang, China). Novozym 435 (lipase B from *Candida antarctica*, immobilized on a macroporous acrylic resin), Lipozyme 435 (a recombinant lipase from *C. antarctica*, expressed on *Aspergillus niger*, and immobilized on Lewatit VP OC 1600), Lipozyme RM IM (lipase from *Rhizomucor miehei*, immobilized on an anionic exchange resin), and Lipozyme TL IM (lipase from *Thermomyces lanuginosus*, immobilized on silica granulation) were obtained from Novozymes (Beijing, China). Phytosterol (62.4% β-sitosterol, 14.7% stigmasterol, 20.6% campesterol, 2.2% brassicasterol, 410.8 g/mol average molecular weight) was provided by Lantian Bioengineering Co., Ltd (Xi'an, China). A standard mixture containing α-, β-, γ-, and δ-tocopherols and cholesteryl stearate standard were purchased from Sigma-Aldrich Chemical Co. (Shanghai, China). A standard mixture containing α-, β-, γ-, and δ-tocotrienols was obtained from Beijing Gingko-Group Biological Technology Co., Ltd (Beijing, China). A 4-Å molecular sieve (activated in a muffle furnace at 550 ± 10 °C for 2 h) and organic solvents were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

2.2. Lipase-catalyzed deacidification

A reaction mixture containing 2.34 g of phytosterol (1:1 molar ratio of FFA to phytosterol), 10 g of HRBO, organic solvent, a 4-Å molecular sieve, and 5% (relative to total weight of substrates) lipase were stirred (about 200 rpm) at a controlled temperature in a 100-mL three-necked round-bottom flask with condensation reflux. The reaction time was varied in the range of 12–72 h when the reaction time factor was optimized. When a solvent-free system (0 mL of solvent) was used, the esterification was conducted under vacuum (8 mmHg) without the addition of a molecular sieve. Lipase was used as a catalyst to initiate the esterification reaction. At the end of the reaction, the lipase and molecular sieve were removed by centrifugation, and the organic solvent was evaporated under reduced pressure. Subsequently, the resulting mixture was centrifuged to remove the unreacted phytosterol after being held at 20 °C for 1 h. All reactions were run in duplicate. The FFA conversion was calculated as follows:

$$C = \frac{C1 - C2}{C1} \times 100\%$$

C, FFA conversion.

C1, FFA amount (w/w, relative to total mass) of HRBO.

C2, FFA amount (w/w, relative to total mass) of deacidified RBO.

2.3. Comparative study of oxidation stability of HRBO, CRBO, and deacidified RBO

The Schaal oven-accelerated oxidation method and the Rancimat test were used to evaluate the oxidation stability [23,24]. For the evaluation of oxidation stability by the Schaal oven-accelerated oxidation method, 100 g of HRBO, CRBO, and deacidified RBO were weighed in test tubes at 63 ± 0.5 °C in the dark in a shaking water bath, respectively. Samples were periodically taken for the determination of vitamin E and peroxide level. The oxidative stabilities of HRBO, CRBO, and deacidified RBO were also compared using the Rancimat method operated at 110, 120, 130, and 140 °C. Namely, 3-g samples were placed in every test tube. The flow rate of air passing through the heated oils was set at 20 L/h. The stability of the RBO samples can be evaluated based on the induction time.

2.4. Analysis

2.4.1. Analysis of vitamin E

Analysis of vitamin E was performed using high-performance liquid chromatography (HPLC) as described previously [25]. A Waters 2996 Diode array detector (DAD) chromatographic system equipped with an ultraviolet (UV) detector set at 295 nm was used. The used column was Waters Spherisorb Silica (50 × 4.6 mm, 5 μm). The column temperature was set at 35 °C. The samples were eluted with hexane/isopropanol (98.5:1.5, v/v) at 0.8 mL/min flow rate. A standard mixture of α-, β-, γ-, and δ-tocopherols and a mixture of α-, β-, γ-, and δ-tocotrienols were used as external standards to identify the peaks based on the HPLC retention times.

2.4.2. Determination of the FFA and peroxide value

The FFA content of RBO and peroxide value were determined according to American Oil Chemists' Society (AOCS) official methods Ca 5a-40 and Cd 8b-90, respectively.

2.4.3. Determination of phytosterol and phytosterol ester

For the quantification of phytosterol ester, HPLC-evaporation light scattering detector (ELSD) using a Waters 1525 liquid chromatographic system (Waters Corp., Milford, MA, USA) equipped with a LiChrospher Si column (250 mm × 4.6 mm, 5-μm particle size, Sigma–Aldrich Corp. K.K., Tokyo, Japan) was chosen, and the products were eluted with a binary gradient of solvent A (100% of hexane) and solvent B (1:1:0.01 isopropanol/hexane/acetic acid, v/v/v) at 1.0 mL/min. Samples were diluted to 1 mg/mL and analyzed according to the following gradient profile: solvent A was decreased from 100% to 90% over 10 min and decreased further to 80% from 10 to 20 min. Finally, solvent A was held at 80% for 5 min. The total run time was 25 min.