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In situ lipase-catalyzed transesterification in rice bran for synthesis of fatty acid methyl ester

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ABSTRACT

Fatty acid methyl ester (FAME) were synthesized via *in situ* lipase-catalyzed transesterification in rice bran without additional catalyst. With this method, FAME were synthesized directly from oil in rice bran by simply adding methanol, with the aid of rice bran lipase already existing in rice bran. The effects of temperature, molar ratio (oil in rice bran to methanol), and water content of the rice bran were investigated. The yield of FAME and the free fatty acid content were monitored as a function of reaction time. The optimum conditions were a temperature of 40 °C, a molar ratio of 1:6, and a water content of 12%. Under the optimum conditions, the FAME yield of 83.4 wt% was obtained after 12 days. To further increase the FAME yield, the transesterification was repeated using the rice bran obtained from the first transesterification. The oils in rice bran that could be converted to FAME were completely transformed throughout the repeated transesterification.

1. Introduction

Biodiesel is defined as monoalkyl esters of long-chain fatty acids derived from vegetable oils or animal fats for use in diesel engines (Bisen et al., 2010; Robles-Medina et al., 2009). It has become an important alternative to conventional diesel fuel since it is renewable, biodegradable, non-toxic, and essentially free of sulfur and aromatics. The use of biodiesel can reduce the exhaust emissions of particulate matters and green-house gases (Körbitz, 1999).

The fundamental reaction in biodiesel production is transesterification, which can be catalyzed either chemically or enzymatically. Short reaction time and high yield of biodiesel are advantages of chemical transesterification (Marchetti et al., 2007). However, it has several major disadvantages, namely, high-energy requirements, difficulties in the recovery of catalysts and glycerol, and environmental pollution associated with the generation of large volumes of wastewater (Ognjanovic et al., 2009; Salis et al., 2005). Enzymatic transesterification can overcome the problems facing conventional chemical methods without compromising their advantages. Most importantly, enzymatic transesterification is carried out under mild reaction condition, and does not require any complex process for glycerol recovery or wastewater treatment (Al-Zuhair et al., 2007). Thus, enzymatic transesterification has been widely studied for the biodiesel production. However, high cost and short life span of enzymes are major drawbacks of enzymatic methods. Supercritical technology has emerged as a good alternative method for biodiesel production, since supercritical alcohols can react with refined oils efficiently without a help of catalyst (Rathore and Madras, 2007; Saka and Kusdiana, 2001). However, this method is economically inefficient due to the high-energy requirements.

In situ transesterification is another effective method for the production of biodiesel. In this method, oil is not extracted from seeds or oil-bearing sources prior to the transesterification. Instead, the oil-bearing material contacts with alcohol directly in the presence of a catalyst. For the catalyst, chemical catalysts such as sulfuric acid or sodium hydroxide have been commonly used for biodiesel production under *in situ* condition (Georgogianni et al., 2008; Harrington and D'Arcy-Evans, 1985). Several studies have investigated the synthesis of biodiesel using rice bran as a substrate via *in situ* transesterification with a chemical catalyst (Lei et al., 2010; Özgül-Yücel and Türkay, 2003; Shiu et al., 2010).

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Rice bran is a by-product obtained from the outer layer of the brown rice kernel during milling to produce white rice. It contains 12–23% crude oil, which mainly consists of triacylglycerol (TAG), depending on the rice origin (Luh et al., 1991). Immediately following the milling process, rice bran deteriorates rapidly because the lipase in the rice bran has high hydrolytic activity. Free fatty acid (FFA) resulted from hydrolysis of TAG increase acidity which contributes to the formation of an off-flavor and soapy taste, and changes in functional property. The increase in FFA is unprofitable for the oil extraction in the industrial perspective due to high refining loss (Tao et al., 1993). Thus, to prevent hydrolysis caused by rice bran lipase, stabilization of rice bran immediately after the milling has been necessary but arduous task for oil industry. Numerous researches on rice bran lipase focused on its deactivation to stabilize the rice bran. Various technologies such as heating, treatment with hydrochloric acid, exposure to microwave irradiation, treatments with chemical inhibitors, low temperature storage, and osmic heating, have been explored for deactivation of the rice bran (Prabhakar and Venkatesh, 1986; Prakash and Ramanatham, 1994; Raghavendra et al., 2007). However, the possible utilization of rice bran lipase for beneficial purposes has not been studied.

The aim of this study was to investigate *in situ* lipase-catalyzed transesterification in rice bran for the production of fatty acid methyl ester (FAME). Surprisingly, FAME were synthesized effectively with methanol by a lipase naturally existing in the rice bran. For optimization of the reaction, the effects of temperature, molar ratio (oil in rice bran to methanol), and water content were thoroughly studied. In addition, the effect of repeated transesterification was also investigated.

2. Materials and methods

2.1. Materials

The rice bran used in this study was supplied by the Korean Rural Development Administration (Suwon, Republic of Korea) and the rice cultivar was *Boramchan*. The rice bran prior to use was passed through a 30-mesh sieve to sift out the hull, germ, broken endosperm part, and any other impurities. The oil and water contents of rice bran were 15 wt %, and 12%, respectively. The oils in rice bran were composed of 0.7 wt % of FFA, 1.8 wt% of diacylglycerol (DAG), 92.7 wt% of TAG, and 4.8 wt% of unsaponifiable matters. The fatty acids in crude rice bran oil consisted of 0.2 wt% myristic acid, 16.5 wt% palmitic acid, 0.1 wt% palmitoleic acid, 1.4 wt% stearic acid, 42.2 wt% oleic acid, 39.1 wt% linoleic acid, and 0.5 wt% of eicosenoic acid. The rice bran was stored in sealed containers at −85 °C until used. Tricaprin (GLC-570) as an internal standard was purchased from Nu-Check Prep, Inc. (Elysian, MN, USA) and fatty acid methyl ester mixture (CRM47885) was purchased from Sigma Aldrich Korea (Seoul, Republic of Korea). The other chemicals used in this study were of analytical grade unless otherwise noted.

2.2. Effect of heat treatment on the catalytic activity of rice bran lipase

To verify synthesis of FAME by *in situ* lipase-catalyzed transesterification in rice bran, the rice bran was heated for 15 min, 30 min, and 60 min and then divided into two groups to investigate the transesterification activity for the synthesis of FAME (first group) and the hydrolytic activity for the formation of FFA (second group). As a control, unheated rice bran was incubated in the same manner. The heat treatment was carried out with rice bran sealed in a glass bottle. After the heat treatment, the rice bran was employed for determination of hydrolytic and transesterification activities.

The heated rice bran as first group was incubated with the addition of methanol to investigate the transesterification activity. The incubation temperature, the molar ratio of the oil in rice bran to methanol, and the water content of the rice bran were set at 40 °C, 1:6, and 12%, respectively. The heated rice bran as second group was incubated without the addition of methanol to investigate the hydrolytic activity. The reaction was performed without methanol and other conditions were the same as those of first group. The yield of FAME and FFA content were investigated for the transesterification activity and hydrolytic activity, respectively.

2.3. In situ lipase-catalyzed transesterification in rice bran

For *in situ* lipase-catalyzed transesterification in rice bran, rice bran (100.0 g), which contains 15.0 g of oil (17.3 mmol, based on TAG), was put into a 1 L polypropylene bottle and 3.3 g of methanol (103.7 mmol) was added. The mixture of rice bran and methanol was then shaken vigorously for 5 min until the rice bran was uniformly wetted by the methanol. The rice bran wetted with the methanol was divided in a 15 mL polypropylene bottle and each bottle was tightly closed with a screw cap to prevent methanol from leaking. These samples were incubated in an incubator (Model IB-15G; Jeio tech, Daejeon, Republic of Korea) at the desired temperature and samples in individual bottles were taken for analysis at the desired reaction time. Subsequently, the oils in the rice bran were extracted in a 250 mL flask by stirring with 100 mL of *n*-hexane for 1 h. The extraction was performed twice and *n*-hexane was completely removed using a rotary evaporator at 60 °C. The oils were stored in a glass vial at −85 °C until used.

The effects of temperature (20–70 °C), molar ratio of oil in rice bran to methanol (1:3-1:9), and water content (0–24%) on the *in situ* lipase-catalyzed transesterification in rice bran were investigated. Because the rice bran initially had a water content of 12%, samples containing 0% and 6% water were prepared by removing some of the water by freeze-drying. Samples with water contents of 18% and 24% were prepared by adding appropriate volumes of water to the rice bran. The water content of rice bran was determined by the oven drying method at 105 °C according to AOAC Official Method 950.01 (2002).

2.4. Analysis of products

Fifty milligram of oil extracted was weighed accurately into a 5 mL volumetric flask and diluted with chloroform. Each sample solution was transferred to a vial and analyzed by a gas chromatography. Tricaprin (0.5 mg/mL) was used as an internal standard. A gas chromatography (Model 3800; Varian, Palo Alto, CA, USA) equipped with a DB-1ht capillary column (15 m × 0.25 mm i.d.; J&W Scientific, Folsom, CA, USA) and a flame ionization detector was used. The column was initially held at 120 °C for 3 min and then heated to 370 °C at a rate of 20 °C/min. The column was then held at 370 °C for 3 min. Helium was used as a carrier gas at a flow rate of 1.5 mL/min and a split ratio was 1/50. The injector and detector temperatures were set at 370 °C.

The yield of FAME (wt%) was calculated as in the following equation.

$$\text{Yield of FAME (wt\%)} = a/b \times 100$$

Where a is the weight of FAME in rice bran, and b is the total weight of FAME, FFA, monoacylglycerol (MAG), DAG, TAG, and unsaponifiable matters in rice bran. FFA content was measured by acid value according to AOAC Official Method 940.28 (2002) and the content of unsaponifiable matters was determined according to AOAC Official Method 933.08 (2002). All experiments were conducted in triplicate.

3. Results and discussion

3.1. Effect of heat treatment on the catalytic activity of rice bran lipase

Rice bran lipase is known to have strong hydrolytic activity, which increases the FFA content of rice bran and decrease the oil extraction recovery. More than two types of the rice bran lipases have been identified, and they tend to have a regiospecificity at *sn*-1, 3 positions of