Lipase-Catalyzed Modification of Rice Bran Oil To Incorporate Capric Acid

Brenda H. Jennings and Casimir C. Akoh*

Department of Food Science and Technology, Food Science Building, The University of Georgia, Athens, Georgia 30602-7610

Capric acid (C10:0) was incorporated into rice bran oil with an immobilized lipase from *Rhizomucor miehei* as the biocatalyst. Effects of incubation time, substrate mole ratio, enzyme load, and water addition on mole percent incorporation of C10:0 were studied. Transesterification was performed in an organic solvent, hexane, and under solvent-free condition. Pancreatic lipase-catalyzed *sn*-2 positional analysis and tocopherol analysis were performed before and after enzymatic modification. Products were analyzed by gas−liquid chromatography (GLC) for fatty acid composition. After 24 h of incubation in hexane, there was an average of 26.5 ± 1.8 mol % incorporation of C10:0 into rice bran oil. The solvent-free reaction produced an average of 24.5 ± 3.7 mol % capric acid. In general, as the enzyme load, substrate mole ratio, and incubation time increased, the mole percent of capric acid incorporation also increased. Time course reaction indicated C10:0 incorporation increased up to 27.0 mol % at 72 h, for the reaction in hexane, and up to 29.6 mol % at 12 h, for the solvent-free reaction. The highest C10:0 incorporations (53.1 and 43.2 mol %) for the mole ratio experiment occurred at a mole ratio of 1:8 for solvent and solvent-free reactions, respectively. The highest C10:0 incorporation (27.9 mol %) for the reaction in hexane occurred at 10% enzyme load, and the highest incorporation (34.4 mol %) for the solvent-free reaction occurred at 20% enzyme load. Incorporation of C10:0 into rice bran oil declined with the addition of increasing amounts of water after reaching 30.3 mol % at 2% water addition in hexane, and in the solvent-free reaction after reaching 35.9 mol %.

Keywords: *Acidolysis; capric acid; immobilized lipase; IM60; Rhizomucor miehei; rice bran oil; structured lipid; transesterification*

INTRODUCTION

Rice bran oil is a byproduct of rice milling and is consumed widely in Asia (Sharkar and Bhattacharyya, 1991). There has been much interest in rice bran oil in recent years because it contains tocotrienols, sterols, and γ-oryzanol, which have been found to lower serum cholesterol (Rukmini and Raghuram, 1991). Rice bran oil contains ~38% oleic acid and ~34% linoleic acid (Rukmini and Raghuram, 1991).

Fat absorption abnormalities occurring in diseases such as cystic fibrosis, colitis, and that which occurs in premature infants can cause deficiencies in essential fatty acids and in fat-soluble vitamins (Kennedy, 1991). Medium-chain fatty acids (MCFA) such as capric acid (C10:0) are absorbed more easily than long-chain fatty acids (LCFA) by patients with fat absorption abnormalities and can help provide proper nourishment for such individuals (Rukmini and Raghuram, 1991). Medium-chain triacylglycerols (MCT) are of interest for those with health and fitness concerns because MCT are utilized rapidly for energy and not normally deposited in adipose tissue as fat. MCT also have been used in high-energy diets for patients and athletes (Megremis, 1991) who need a rapid source of energy. However, MCT could not function as the only source of dietary fat because they do not provide essential fatty acids required for human health. A structured lipid that contained both rice bran oil and C10:0 fatty acids on the same glycerol backbone would have the combined health benefits of both and may help to alleviate fat absorption abnormalities.

Transesterification reactions catalyzed by chemicals or enzymes produce structured lipids by changing the fatty acids or stereospecific distribution of fatty acids in the glycerol molecule (Akoh, 1995). In this study, the fatty acid content of rice bran oil was modified to contain C10:0 by using an immobilized lipase, IM60, from *Rhizomucor miehei*, as the biocatalyst. The objective of this study was to determine the effects of incubation time, substrate mole ratio, enzyme load, water addition, and solvent versus solvent-free reactions on the mole percent incorporation of C10:0.

MATERIALS AND METHODS

Materials. Rice bran oil Loriva Supreme Foods, Inc. (Ronkonkoma, NY), was purchased from a local supermarket. Immobilized 1,3 specific lipase, IM60 (Lipozyme IM), was provided by Novo Nordisk Biochem North America, Inc. (Franklinton, NC). Capric acid was obtained from Sigma Chemical Co. (St. Louis, MO). Organic solvents were purchased from J. T. Baker Chemical Co. (Phillipsburg, NJ) or Fisher Scientific (Norcross, GA).

Enzymatic Modification Reaction. For general modification of rice bran oil, 100 mg of rice bran oil was mixed with C10:0 (39.4 mg) at a mole ratio of 1:2 triacylglycerol to C10:0 free fatty acid in 3 mL of *n*-hexane. Immobilized IM60 lipase was added at 10 wt % of reactants and the mixture incubated

* Author to whom correspondence should be addressed [telephone (706) 542-1067; fax (706) 542-1050; e-mail cmscakoh@arches.uga.edu].

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in an orbital shaking water bath at 55 °C for 24 h at 200 rpm. All reactions were performed in duplicate, and mean values are reported.

Analysis of Product. The enzyme and any residual moisture were removed by passing the reaction mixture through an anhydrous sodium sulfate packed in a Pasteur pipet column. A 50-μL aliquot of the reaction product was analyzed by thin-layer chromatography (TLC) on silica gel 60 plates developed with petroleum ether/ethyl ether/acetic acid (80:20:0.5, v/v/v). The bands were visualized under ultraviolet light after being sprayed with 0.2% dichlorofluorescein in methanol. Bands containing modified triacylglycerols (TAG) were scraped from the TLC plate and methylated with 3 mL of 6% HCl in methanol at 75 °C for 2 h (Jennings and Akoh, 1999). The fatty acid methyl esters (FAME) were extracted twice with 1 mL of hexane, dried over sodium sulfate, and concentrated under nitrogen. The reaction product was then analyzed by gas−liquid chromatography (GLC). The injector and detector temperatures were 250 and 260 °C, respectively. The column temperature was held isothermally at 205 °C with helium as the carrier gas. The relative content of FAME as mole percent was calculated by computer with 17:0 as the internal standard.

Pancreatic Lipase-Catalyzed *sn*-2 Positional Analysis. Fifty microliters of the reaction mixture was spotted onto a silica gel 60 TLC plate and developed (as described under Analysis of Product). The band corresponding to TAG was scraped from the TLC plate, extracted twice with ethyl ether, and passed through a sodium sulfate column. The ethyl ether was then evaporated under nitrogen. One milliliter of Tris buffer (pH 7.6), 0.25 mL of bile salts (0.05%), 0.1 mL of $CaCl_2$ (2.2%), and 8.0 mg of purified pancreatic lipase were added to the reaction mixture (Luddy et al., 1963). The mixture was then incubated for 3 min at 37−40 °C, extracted two times with ethyl ether, evaporated under nitrogen, and brought to a final volume of 200 μL. The band corresponding to *sn*-2 monoacylglycerol (2-monoolein was used as a standard) was scraped after the TLC plate was developed in hexane/diethyl ether/acetic acid (50:50:1.0, v/v/v). The *sn*-2 monoacylglycerol was then methylated and analyzed by GLC.

Tocopherol Analysis. Tocopherol analysis was performed on the rice bran oils before and after enzymatic modification. The unmodified oil was diluted 0.1 g of oil to 25 mL of hexane. Enzymatically modified oil was filtered through a sodium sulfate column to remove the enzyme and diluted (1.0 mL of modified oil reaction mixture to 25 mL of hexane). Tocopherol standards were obtained from Sigma (St. Louis, MO). The purity and stability of standards were monitored by specific absorption coefficients ($E^{1\%}_{1cm}$) values measured by using a DU-64 spectrophotometer (Beckman Instruments Inc., Fullerton, CA). Specific $E^{1\%}_{1cm}$ and maximum wavelengths λ_{max} for tocopherols were 71.0 and 294 for α-tocopherol, 86.4 and 297 for β-tocopherol, 92.8 and 298 for γ-tocopherol, and, 91.2 and 298 for δ-tocopherol, respectively (Scott, 1978). Twenty-five milligrams of each homologue was accurately weighed and dissolved in 25 mL of hexane containing butylated hydroxytoluene (BHT; 0.01%, w/v). Appropriate dilutions were made with the mobile phase to give stock standard concentrations of 18.54, 0.51, 20.70, and 3.30 μg/mL for α-, β-, γ-, and δ-tocopherol, respectively. For daily working standard, 2 mL of the stock standard solution was diluted into a 50-mL volumetric flask with mobile phase. The working standard concentrations of α-, β-, γ-, and δ-tocopherol were 0.74, 0.02, 0.82, and 0.14 μg/mL, respectively. Concentrations of the tocopherol homologues were calculated from peak area determined by the Waters 764 integrator (Millipore Corp., Cary, NC). Peak responses for tocopherols were used to quantify the corresponding tocotrienols (Thompson and Hatina, 1979). The oil samples were then analyzed on a normal phase high-performance liquid chromatograph (HPLC) system equipped with a Shimadzu LC-6A pump and an RF-10A spectrofluorometric detector (Shimadzu Corp., Kyoto, Japan), a Spectra series AS100 autosampler (Thermo Separation Products Inc., San Jose, CA), and a 25 cm × 4 mm, 5-μm Lichrosorb Si 60 column (Hibar Fertigsaub RT, Darmstadt, Germany) equipped with a precolumn packed with Perisorb A 30−40 μm (Hibar Fertigsaub RT). The isocratic mobile phase contained 0.6% 2-propanol in *n*-hexane (J. T. Baker Chemical Co., Phillipsburg, NJ), and the flow rate was 1.0 mL/min. The mobile phase was filtered with a 0.22 μm nylon filter (MSI Inc., Westboro, MA) and degassed by stirring under vacuum. The wavelengths were set at 290 nm for excitation and at 330 nm for emission for the determination of tocopherol and tocotrienol homologues (Lee et al., 1999).

Table 1. Fatty Acids (Mole Percent) in Rice Bran Oil before and after Enzymatic Modification with and without Hexane

fatty acid	before modification	after modification	
		n-hexane	solvent-free
10:0	0.0 ± 0.0	26.5 ± 1.8	24.5 ± 3.5
16:0	13.5 ± 3.2	11.2 ± 4.2	10.8 ± 0.9
18:0	3.4 ± 1.1	1.7 ± 1.2	0.3 ± 0.6
18:1*n*−9	48.9 ± 0.3	37.2 ± 2.0	37.8 ± 3.1
18:2*n*−6	32.4 ± 1.3	27.5 ± 4.4	27.4 ± 2.9
18:3*n*−3	1.9 ± 0.5	ND[a]	ND

[a] ND, not detected.

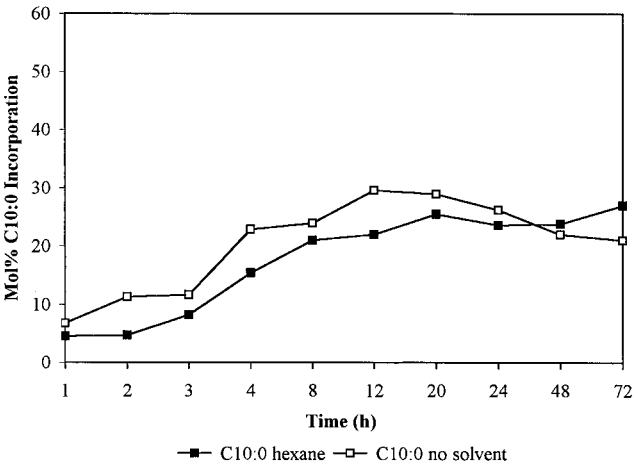

Figure 1. Time course of IM60 lipase catalyzed modification of rice bran oil with and without hexane to incorporate capric acid (C10:0) as determined by GLC. Samples were analyzed at 1, 2, 3, 4, 8, 12, 20, 24, 48, and 72 h (*x*-axis). All reactions were in duplicate. Enzyme amount was 10 wt % reactants. Incubation was at 55 °C and 200 rpm with and without hexane. The *y*-axis represents the mole percent of C10:0 incorporation.

RESULTS AND DISCUSSION

The fatty acid compositions of rice bran oil before and after modification show that C10:0 was successfully incorporated into rice bran oil (Table 1). After 24 h of incubation in hexane at a substrate mole ratio of 1:2, there was an average of 26.5 ± 3.5 mol % incorporation of C10:0 into rice bran oil or 39.8% theoretical incorporation (66.6% is the maximum expected). The solvent-free reaction produced an average mole percent incorporation of 24.5 ± 3.7 mol %, which represents 36.8% maximum incorporation. Generally, as enzyme load, substrate mole ratio, and incubation time increased, mole percent incorporation of C10:0 also increased (Figures 1−3). These results were consistent with other studies involving the enzymatic modification of triacylglycerols from our laboratory (Akoh et al., 1995, 1996).

Time Course. During the time course reaction in hexane (Figure 1), C10:0 incorporation increased for up

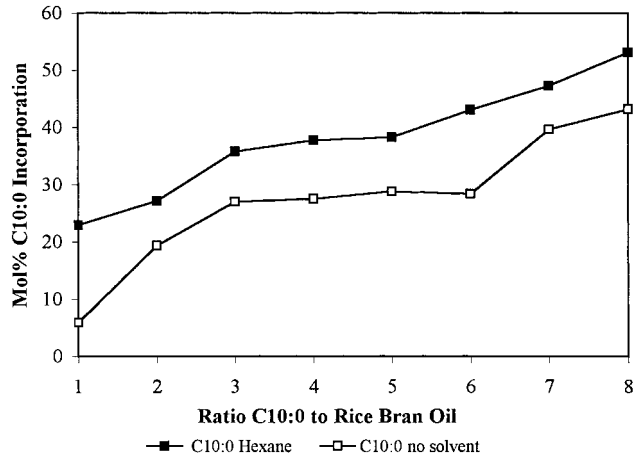

Figure 2. Effect of substrate mole ratio (rice bran oil to C10:0) on C10:0 incorporation with IM60 lipase as biocatalyst. Numbers 1−8 on the *x*-axis correspond to mole ratios of 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, and 1:8, respectively. All reactions were in duplicate. Enzyme amount was 10 wt % reactants. Incubation was at 55 °C and 200 rpm with and without hexane. The *y*-axis represents the mole percent of C10:0 incorporation.

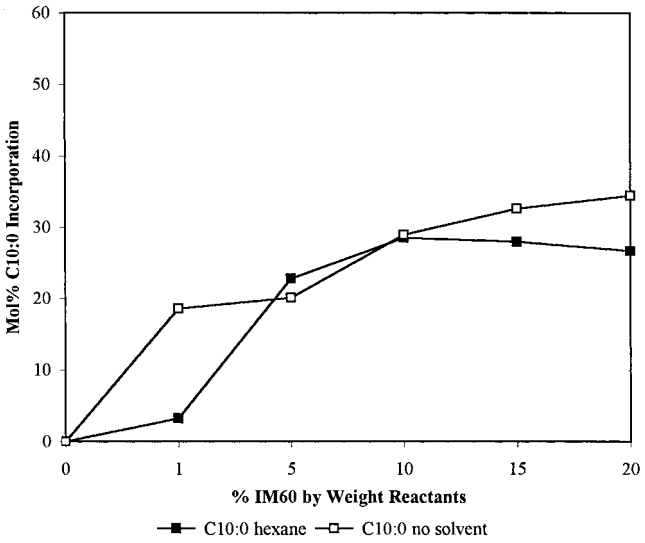

Figure 3. Effect of enzyme load on C10:0 incorporation into rice bran oil with IM60 lipase as biocatalyst. The amount of enzyme was based on the weight of reactants (0, 5, 10, 15, and 20%). All reactions were in duplicate. Incubation was at 55 °C and 200 rpm with and without hexane. The *y*-axis represents the mole percent of C10:0 incorporation.

to 72 h to 27.0 mol % but declined after an optimum at 12 h from 29.6 mol % for the solvent-free reaction. Others have reported that longer reaction times result in higher incorporation of MCFA but also lead to increased acyl migration in a laboratory scale continuous reactor when MCFA were incorporated into TAG with IM60 as a biocatalyst (Mu et al., 1998).

Substrate Mole Ratio. The highest value for C10:0 incorporation was 53.1% and was achieved at a substrate mole ratio of 1:8 TAG to C10:0 in hexane (Figure 2). For solvent-free reactions, the mole percent incorporation followed similar trends, with the highest C10:0 incorporation at 43.2 mol %, which occurred at a mole ratio of 1:8. The lower incorporation in the solvent-free system could have been due to increased viscosity and mass transfer limitations. Previous studies have shown that a high substrate mole ratio required a shorter reaction time, led to improved reaction rate, and resulted in less acyl migration (Mu et al., 1998). There is no economic advantage to using excess capric acid, which is expensive. Excess capric acid can also cause lipase inhibition. Product yield can also decrease with downstream processing to remove excess C10:0 when the modification is carried out in large scale.

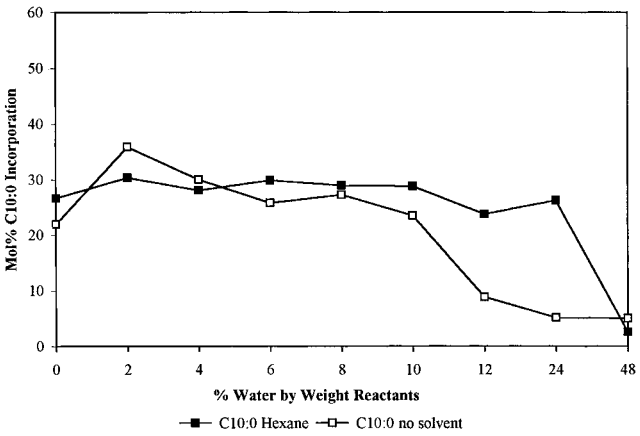

Figure 4. Effect of water addition on C10:0 incorporation into rice bran oil with IM60 lipase as biocatalyst. The amount of water added was based on the weight of the reactants (0, 1, 2, 4, 6, 8, 10, 12, 24, and 48%, respectively). All reactions were in duplicate. Incubation was at 55 °C and 200 rpm with and without hexane. The *y*-axis represents the mole percent of C10:0 incorporation.

Enzyme Load. The optimum C10:0 incorporation (27.9 mol %) occurred at 10 wt % of enzyme in hexane and at 20% enzyme (34.2 mol %) for the solvent-free reaction (Figure 3). Others have also reported increased incorporation of capric acid into rapeseed oil with increasing enzyme load; however, acyl migration also increased (Xu et al., 1998b). The cost of substrates, enzymes, energy, and manpower must be taken into account in the determination of the most economic method of producing the desired product. Increased enzyme load only accelerates the reaction rate.

Effect of Water Addition. There was a decline in mole percent incorporation of C10:0 with increasing amounts of water (0−12%) for the solvent-free reactions (Figure 4). The water content of the unmodified rice bran oil was 0.07%. The optimum C10:0 incorporation occurred at 2% water for reactions with (30.3 mol %) and without (35.9 mol %) hexane; afterward, C10:0 incorporation declined to 5 mol % or less. The change was due to the acidolysis reaction shifting in the direction of hydrolysis rather than synthesis when excess water was added. For most reactions, a water content of <1% is required, but the optimum water content varies between 0.04 and 11% for different lipases (Malcata et al., 1992; Li and Ward, 1993) and must be determined on a case by case basis.

Solvent versus Solvent-Free Reaction. Organic solvents such as hexane have several functions, including increasing the solubility of nonpolar substrates and shifting the thermodynamic equilibria to synthesis rather than hydrolysis (Klibanov, 1986). Concerns for hexane toxicity, flammability, cost, and additional time associated with the purification process have led to lipase-catalyzed modification of TAG being performed without organic solvent (green reaction). Mole percent incorporations of C10:0 were comparable for the time course reaction and for the effect of water reaction up to 10% water. For the enzyme load reaction, mole percent incorporation was slightly higher than or equal

Table 2. Tocopherol and Tocotrienol Contents of Rice Bran Oil before and after Enzymatic Modification

	tocopherol content (mg/g)					tocotrienol content (mg/g)				
sample	α	β	γ	δ	total	α	β	γ	δ	total
unmodified oil	0.1303	0.0028	0.1016	0.0058	0.2405	0.0519	ND[a]	0.1188	0.0040	0.1747
modified oil	0.1080	0.0030	0.1020	ND	0.2130	0.0420	ND	0.1110	ND	0.1530

[a] ND, not detected.

to values for the reaction in hexane. The mole ratio experiment showed higher mole percent C10:0 incorporation for the reaction in hexane than the solvent-free reaction. Enzymatic lipid modification reactions in solvent-free media are preferred over chemical modification or reactions in organic solvents for food applications.

Pancreatic Lipase *sn*-2 Positional Analysis. Pancreatic lipase-catalyzed *sn*-2 positional analysis of rice bran oil before and after modification showed the following fatty acid composition: C10:0, 0.0 and 12.4 mol %; C18:1, 42.7 and 46.8 mol %; C18:2, 57.3 and 40.8 mol %, respectively. Of the total fatty acids at the 2-position, C10:0 represents 14.2%, and the remaining 85.8% of the modified rice bran oil is of MLM type (M = medium-chain, C10:0; and L = long-chain fatty acids, mainly C18:2 and C18:1). Unsaturated fatty acids, especially linoleic acid, are preferentially placed at the 2-position in oil seeds (Nawar, 1985). The presence of 10:0 at the 2-position, even though a 1,3 specific lipase was used, demonstrates some acyl migration. Others have reported that in addition to reaction time, water content, lipase load, temperature, acyl donor type, and lipase type also influence acyl migration (Xu et al., 1998a). Over 40 mol % of the essential fatty acid C18:2 was at the 2-position after modification. Fatty acids esterified at the 2-position are easily absorbed, regardless of the type of fatty acid esterified at that position (Haumann, 1997; Jandacek et al., 1987).

Tocopherol Analysis. Tocopherols are found naturally in oils, function as antioxidants, and offer several health benefits. Table 2 shows the tocopherol and tocotreinol contents of rice bran oil before and after enzymatic modification. Tocopherols detected were α-, β-, δ-, and γ-. The amount of tocopherols and tocotreinols seemed to change by only a small amount after modification. Rice bran oil has been reported to contain 0.550 mg/g total tocopherols with 0.181 mg/g tocopherols and 0.369 mg/g tocotrienols (Rukmini and Raghuram, 1991). The rice bran oil tested contained 0.2405 mg/g tocopherols before modification and 0.2130 mg/g after modification. The level of tocotrienols in rice bran oil was also measured (Table 2) to determine the effect of enzymatic modification because tocotrienols play a role in the cholesterol-lowering effects of rice bran oil (Rukmini and Raghuram, 1991). The rice bran oil tested contained 0.1747 mg/g α-, δ-, and γ-tocotrienols before modification and 0.1530 mg/g total α- and γ-tocotrienol after modification. However, it should be noted that δ-tocopherol and δ-tocotrienol completely disappeared after modification. These were probably lost during enzymatic modification and further processing. Exposure to light may have contributed to the loss. We reported substantial decreases in α-, β-, and γ-tocopherol contents of enzymatically synthesized fish oil-based structured lipids (Jennings and Akoh, 1999). The current finding suggests that rice bran oil may retain many of its health benefits and oxidative stability after enzymatic modification. Rice bran oil containing capric acid was successfully produced and may be more beneficial for certain applications than unmodified oil. The benefits of a C10:0 and rice bran oil structured lipid may be in health and disease prevention with fewer calories and less fat storage in the adipose tissue.

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