

Characterization of a Rice Bran Oil Structured Lipid

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Rice bran oil (RBO) was enzymatically modified in a continuous packed bed bioreactor to incorporate caprylic acid with Lipozyme RM IM as biocatalyst. The reaction product was purified by short-path distillation. Rice bran oil structured lipid (RBOSL) contained 32.1 mol % caprylic acid. Positional analysis revealed 0.7 mol % caprylic acid at the sn-2 position and 47.8 mol % caprylic acid at the sn-1,3 positions. Composition of free fatty acids and smoke point of RBO and RBOSL were not significantly different. Saponification value, iodine value, and viscosity of RBO were significantly different from those of RBOSL. The color of RBOSL was darker, more yellow and less green than RBO. Volatile compounds in RBO and RBOSL were determined by GC-MS. Melting onset temperatures of RBO and RBOSL were not significantly different, while melting end point temperatures and melting enthalpies were significantly different. This characterization study results will help determine potential food applications of RBOSL.

KEYWORDS: Caprylic acid; continuous packed bed bioreactor; enzymatic modification; Lipozyme RM IM; rice bran oil; structured lipid

INTRODUCTION

Lipase-catalyzed modification of triacylglycerols (TAG) results in the changes in chemical, physical, and nutritional characteristics of the end product different from the starting triacylglycerols. Characterization of enzymatically modified TAG is necessary to determine potential nutraceutical and food applications. Structured lipids (SL) are defined as TAGs that have been modified by the incorporation of new fatty acids, restructured to change the positions of fatty acids or the fatty acid profile, from the natural state, or synthesized to yield novel TAGs (1). SL components include short chain fatty acids (SCFAs), medium chain fatty acids (MCFAs), and long chain fatty acids (LCFAs). SCFA are lower in calories and more rapidly absorbed than MCFA or LCFA. LCFAs such as linoleic acid are essential fatty acids and are therefore important SL components. MCFAs provide a quick energy source, which can be rapidly oxidized and utilized and are metabolized through the portal system instead of the lymphatic system as are LCFA. MCFAs have been used to treat patients with fat absorption abnormalities and used by athletes with increased energy requirements (2, 3). Previous animal and human studies have shown that composition of TAGs containing MCFA result in increased energy expenditure and decreased weight gain (4, 5). The mobility, solubility, and ease of metabolism of MCFAs provide health benefits when incorporated into SL. Therefore, the characteristic component fatty acids of SL determine the physical properties, digestion, absorption, and metabolism of SL. Improved immune function, reduction in LDL cholesterol,

improved nitrogen balance, and reduction in cancer risk are among the health benefits of SLs (1).

Although a previous study determined the conditions for the enzymatic production of a rice bran oil SL containing caprylic acid (6), additional studies are needed to determine the chemical and physical properties of a rice bran oil SL containing a MCFA to help determine future food applications. A previous study in which sesame oil was modified to contain caprylic acid noted that physical and chemical properties such as viscosity, color, saponification value, iodine value, melting and crystallization behavior, and oxidative stability and volatile compounds were significantly different from those of unmodified sesame oil (7). Another study in which a SL was enzymatically produced with Lipozyme RM IM as biocatalyst from corn oil, capric acid and conjugated linoleic acid found that the iodine value was lower in the SL, while the saponification value was higher in the SL when compared to that of unmodified corn oil (8).

Specific fatty acids can serve as acyl donors and can be transesterified onto specific positions of TAG using sn-1,3 lipases such as Lipozyme RM IM. For this study, rice bran oil was transesterified with the MCFA (caprylic acid) targeted at the sn-1 and sn-3 positions with primarily LCFA oleic and linoleic acids at the sn-2 position. Fatty acids at the sn-1 and sn-3 positions are rapidly hydrolyzed and not stored as fat, whereas fatty acids at the sn-2 position are more readily absorbed (9).

The chemical and physical properties of SL are often different from the unmodified oils from which they are produced, and characterization of these properties is essential in determining possible food applications. The objective of this study was to determine how various chemical and physical properties such

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as fatty acid profile, sn-2 and sn-1,3 positions of fatty acids, smoke point, viscosity, saponification value, iodine value, color, volatile compounds, and melting profiles of a rice bran oil-based SL differ from those of unmodified rice bran oil.

MATERIALS AND METHODS

Materials. Rice bran oil was purchased from California Rice Oil Company (Novato, CA). Caprylic acid was purchased from Sigma Chemical Co. (St. Louis, MO). Lipozyme RM IM (immobilized lipase on a nonporous ion-exchange resin) from *Rhizomucor miehei* was donated by Novo Nordisk Biochem North America, Inc. (Franklinton, NC).

Synthesis of Rice Bran Oil Structured Lipid. The SL was produced in 1 kg quantities in a packed bed reactor with a flow rate of 1 mL/min, 1:6 substrate mole ratio (rice bran oil/caprylic acid), and a temperature of 45 °C (10). A bioreactor with a jacketed stainless steel column (47 mm × 500 mm) and an FMI Laboratory pump model QV from Fluid Metering, Inc. (Oyster Bay, NY) was used for SL synthesis. The bioreactor set up was as reported by Fomuso and Akoh (11). A circulating water bath was used to maintain a constant column temperature. The column was packed with immobilized Lipozyme RM IM and plugged at both ends with approximately 3 cm of glass wool.

Short-Path Distillation. The SL reaction products were passed through a short-path distillation apparatus four times to remove free fatty acids to a level below 0.1%. A KDL-4 (UIC Inc., Joliet, IL) distillation unit was used. The heating oil temperature was 185 °C; the coolant temperature was 15 °C; and the vacuum pressure was below 1 Torr (11).

Gas Liquid Chromatography. Gas liquid chromatography (GLC) was used to determine the fatty acid profiles of rice bran oil SL and the unmodified rice bran oil. The gas chromatograph was an Agilent 6890N (Wilmington, PA) equipped with an AT-225 fused-silica capillary column 30 m × 0.25 mm i.d. (Alltech, Deerfield, IL) and a flame-ionization detector, and operated in splitless mode. The injector and detector temperatures were held at 250 and 260 °C, respectively. The column temperature was at 130 °C for 3 min and then programmed to 215 °C for 20 min at a rate of 20 °C/min. The carrier gas was helium, and the total gas flow rate was 25 mL/min. The relative concentration of FAMES as mol % was calculated by computer with 17:0 as internal standard. Retention times of GLC reference standard (17A prime from Nu-Chek Prep, Inc., Elysian, MN) were used to identify detected FAMES.

sn-2 Positional Analysis. sn-2 positional analysis was conducted on RBO and RBOSL. RBO and RBOSL were spotted onto separate silica gel 60 TLC plates and developed in hexane/ethyl ether/acetic acid (80:20:0.5, vol/vol/vol). The bands corresponding to TAG were scraped from the TLC plate and extracted twice with ethyl ether and passed through a sodium sulfate column. The ethyl ether was then evaporated under nitrogen. One milliliter of 1.0 M Tris buffer (pH 8.0), 0.25 mL of bile salts (0.1%), 0.2 mL of CaCl₂ (22.0%), and 8.0 mg of purified pancreatic lipase were added to the reaction mixture (12). The mixture was then incubated for 3 min at 40 ± 0.5 °C, extracted two times with ethyl ether, evaporated under nitrogen, brought to a final volume of 200 μL, and spotted on a TLC plate. The TLC plate was then developed in hexane/diethyl ether/acetic acid (50:50:1.0, vol/vol/vol) as the developing solvent (12). The bands corresponding to the sn-2 monoacylglycerol (MAG) standard were scraped from the TLC plate. sn-2 MAG was then methylated and analyzed by GLC as previously described.

Differential Scanning Calorimetry. DSC analysis was conducted to compare and differentiate between the RBO and RBOSL melting properties. A Perkin-Elmer model DSC7 (Norwalk, CT) was used for the analysis, which was conducted according to AOCS recommended procedure Cj 1–94 (13). Indium was used as a reference standard and for standardization (mp 156.6, Δ*H* 28.45 J/g), dry ice was used as a coolant. Samples (5–20 mg) were hermetically sealed in 30 μL capacity aluminum pan, and an empty pan was used for reference. Samples were heated rapidly to 80 °C from room temperature for 10 min (to destroy crystal memory). The sample was then cooled to –40 at 10 °C per

min, held for 30 min, then heated to 80 °C at a rate of 5 °C per min to generate melting profiles. The thermograms were then analyzed by the software provided with the DSC (Pyris software, Perkin-Elmer, Shelton, CT).

Volatile Compound Analysis. Volatile compound analysis was conducted on rice bran oil before and after modification to determine the effect of short-path distillation on RBOSL volatile compound concentration. Volatile compounds were extracted by solid phase microextraction (SPME). Samples (25 g) were weighed into 50 mL reaction vials and sealed with Teflon rubber septa. The SPME fiber (polymethylsiloxane/divinylbenzene or PDMS/DVB; blue, Supelco, Inc., Bellefonte, PA) was then inserted into the sample headspace and heated for 1 h at 60 °C to absorb the volatile compounds generated from the samples onto the SPME fiber.

Extracted volatile compounds were desorbed by inserting the SPME fiber into an injection port of a Hewlett-Packard 5890 Series II GC (formerly Hewlett-Packard, Avondale, PA now Agilent, Palo Alto, CA) for 10 min. Desorbed volatile compounds were then separated on a 30 m × 0.25 mm × 0.25 μm EC-WAX capillary column (Alltech, Deerfield, IL). Helium was used as the carrier gas. The splitless mode was used initially during injection for 5 min and then returned to split mode (4.9 mL/min). The initial column temperature was 35 °C for 5 min; the temperature was then increased to 220 °C at the rate of 3 °C/min. The injector temperature was held at 220 °C for 20 min, and the detector temperature was also at 220 °C.

Volatile compounds were identified by gas chromatography–mass spectrometry (GC-MS). GC-MS conditions were the same as those for GC above. Mass spectra were obtained at a MS voltage of 70 eV. The mass range was 35 to 300 (*m/z*). Separated compounds were identified by comparison to mass spectral libraries (National Institute of Standards and Technology, Manchester, U.K. and Wiley Registry, seventh edition, New York, NY).

Other Analytical Methods. The following AOCS official methods were used: Cd 1b-87 for iodine value, Tl 1a-64 for saponification value, Ca 5a-40 for free fatty acid content, and Cc 9a-48 for smoke point (13). Viscosity was determined with an RV Brookfield Digital Viscometer (Brookfield Engineering Laboratories, Inc., Stoughton, MA). Color was measured by a Minolta CR-300 Chroma Meter (Osaka, Japan) using the la Commission Internationale de l'Éclairage (CIE) L*a*b* (for lightness, redness, and yellowness, respectively) color system.

Statistical Analysis. Statistical analysis was performed with the SAS software package (14). A *t*-test was used to determine the differences between two samples (enzymatically modified RBOSL and unmodified RBO). Statistical differences with *P* ≤ 0.05 were considered significant.

RESULTS AND DISCUSSION

Positional Analysis. Unmodified rice bran oil did not contain caprylic acid (Table 1). After modification, the fatty acid profile of RBOSL contained 32.1 ± 0.9 mol% caprylic acid, and the reaction yield was 35.6%. The ratio of saturated to monounsaturated to polyunsaturated fatty acids was 1.6:1.0:1.0. The predominant fatty acids for both RBO and RBOSL at the sn-2 position were oleic and linoleic acids. The small amount of caprylic acid at the sn-2 position for RBOSL indicates that acyl migration was minimal. RBOSL had 47.8 ± 0.9 mol % caprylic acid at the sn-1,3 positions. These results were expected because of the sn-1,3 specific Lipozyme RM IM used for enzymatic modification. A previous enzymatic modification study involving Lipozyme RM IM-catalyzed acidolysis of sesame oil and caprylic acid indicated that a lower temperature, higher substrate mole ratio reduced acyl migration while having a minimal effect on incorporation (10).

Chemical Properties. The free fatty acid (FFA) content of RBOSL was reduced to the level of unmodified RBO after short-path distillation (Table 2). The FFA content of RBOSL after enzymatic modification in the packed-bed reactor was high, and

Table 1. Fatty Acid Composition (mol %) of Total, sn-2, and sn-1,3 Positions of TAG of Rice Bran Oil and Rice Bran Oil Structured Lipid (RBOSL)^a

fatty acid	total		sn-2		sn-1,3	
	rice bran oil	RBOSL	rice bran oil	RBOSL	rice bran oil	RBOSL
caprylic		32.1 ± 0.9		0.7 ± 0.1		47.8 ± 0.9
myristic	0.7 ± 0.0	0.3 ± 0.1				
palmitic	24.8 ± 0.3	11.5 ± 0.3	5.4 ± 0.4	3.7 ± 0.1	34.4 ± 0.1	15.4 ± 0.4
stearic	2.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.3	0.0 ± 0.0	2.8 ± 0.1	0.0 ± 0.0
oleic	37.6 ± 0.1	27.0 ± 0.6	43.0 ± 0.4	43.9 ± 0.1	34.9 ± 0.2	20.4 ± 0.1
linoleic	33.3 ± 0.2	27.1 ± 0.2	50.1 ± 0.2	49.8 ± 0.3	24.9 ± 0.1	15.5 ± 0.1
linolenic	1.2 ± 0.0	0.8 ± 0.0	1.3 ± 0.2	1.2 ± 0.0	1.1 ± 0.0	0.6 ± 0.0
arachidic	0.5 ± 0.0	0.5 ± 0.0				

^a Mean ± SD, $n = 2$. sn-1,3 (mol %) = $[3 \times \text{total (mol \%)} - \text{sn-2 (mol \%)}] / 2$. Structured lipid was prepared by acidolysis of rice bran oil with caprylic acid in a bench scale continuous packed bed reactor.

Table 2. Chemical and Physical Properties of Rice Bran Oil and Rice Bran Oil Structured Lipid (RBOSL)^a

property	rice bran oil	RBOSL
free fatty acid (% oleic)	0.1 ± 0.0 A	0.1 ± 0.0 A
smoke point (°C)	257.0 ± 4.0 A	253.0 ± 3.0 A
saponification value	178.1 ± 0.0 A	206.87 ± 0.7 B
iodine value	92.9 ± 0.2 A	82.2 ± 0.6 B
viscosity (cP)	78.6 ± 0.2 A	66.8 ± 0.2 B

^a Mean ± SD, $n = 2$. Means with the same letter in the same row are not significantly different ($P \leq 0.5$). Structured lipid was prepared by acidolysis of rice bran oil with caprylic acid in a bench-scale packed bed reactor.

this purification step was necessary after the acidolysis reaction to remove FFA and to increase oxidative stability.

The smoke point of RBOSL was not significantly different ($P \geq 0.05$) from that of RBO possibly because the RBO used in this study was refined, bleached, and deodorized (RBD). Refined oils will often have higher smoke points than unrefined oils because they contain less unsaponifiable matter.

The saponification value of RBOSL (206.9 ± 0.7) was significantly higher ($P \leq 0.05$) than RBO (178.1 ± 0.0). The saponification value measures the alkali reactive groups in oils in milligrams of KOH that reacts with 1 g of sample and is an indicator of the molecular weight of the oil. The higher saponification value of RBOSL indicates a lower molecular weight than that of RBO. The lower molecular weight of RBOSL is due to the incorporation of the MCFA, caprylic acid. The saponification value of 178.1 ± 0.0 for RBO was slightly lower than that listed by the Codex Alimentarius Commission (15), which was in the range of 180–195 possibly because of variations in fatty acid composition. The mol % fatty acids for oleic (37.6 ± 0.1) and linoleic acids (33.3 ± 0.2) for the RBO used in this study (Table 1) were slightly lower than or in the lower range of the values listed by the Codex Alimentarius Commission, which were 38–46 total % fatty acids for oleic acid and 33–40 total % fatty acids for linoleic acid (15).

The iodine value of a fat or oil is determined by measuring the amount of iodine that is absorbed per gram of sample and is a measure of the degree of unsaturation. The iodine value for RBO was significantly higher than that for RBOSL. This was because of the incorporation of the saturated fatty acid, caprylic acid, into RBO, which replaced some of the unsaturated fatty acids. The iodine value obtained for RBO in this study was within the range of 90–105 as listed by the Codex Alimentarius Commission (15).

Minor Components and Oxidative Stability. Crude rice bran oil can contain up to 5.4 g/100 mL unsaponifiable matter (which includes tocopherols, sterols, and γ -oryzanol) and may decline to 2.7 g/100 mL after refining, bleaching, and deodorizing (16). A previous study (17) in which rice bran oil was

Table 3. CIE L*a*b* Color of Rice Bran Oil and Rice Bran Oil Structured Lipid (RBOSL)^a

values	rice bran oil	RBOSL
L*	34.5 ± 1.2 A	31.5 ± 1.5 A
a*	-2.8 ± 0.3 A	-2.4 ± 0.0 A
b*	12.2 ± 0.4 A	13.2 ± 1.0 A
C	12.4 ± 0.3 A	13.4 ± 1.2 A
h°	103.2 ± 0.9 A	100.3 ± 0.6 B

^a Mean ± SD, $n = 3$; means with the same letter in the same row are not significantly different ($P \leq 0.05$). Structured lipid was prepared by acidolysis of rice bran oil with caprylic acid in a bench scale continuous packed bed reactor.

enzymatically modified to contain caprylic acid has shown that the total tocopherol and tocotrienol content of refined rice bran oil declined by 43.4% after enzymatic modification and short-path distillation. This study also found that the concentration of γ -oryzanol was not significantly different after enzymatic modification and short-path distillation. The oxidative stability index (OSI) value for RBOSL (11.4 ± 0.0 h) determined in this study was significantly lower ($P \leq 0.05$) than that of RBO (12.4 ± 0.2 h). Another study that determined the characteristics of SL prepared from unrefined sesame oil and caprylic acid found that the concentrations of the tocopherols, phytosterols, and lignins were not significantly different ($P \geq 0.05$) for sesame oil and sesame oil SL, while the oxidative stability of the sesame oil SL was lower (3). The differences in findings may be related to differences in the amount of refining and in the concentrations and types of minor components, and fatty acid compositions of rice bran and sesame oils.

Physical Properties. Viscosity is a measure of a fluid's resistance to flow and was determined for RBO and RBOSL. The viscosity of RBOSL (66.8 ± 0.2 cP) was significantly lower than that of RBO (78.6 ± 0.2 cP) (Table 2). Caprylic acid, which replaced long chain fatty acids, has a lower molecular weight and density than fatty acids present in RBO, and these affect viscosity.

Color. The CIE color system with L* (lightness) a* (redness) and b*(yellowness) showed that the RBO and RBOSL oil samples could be described as dark greenish yellow (Table 3). When L* a* b* values were compared between RBO and RBOSL, RBOSL was darker, less green, and more yellow than RBO. The chroma value (C) also was not significantly different between the oils. The color difference was detectable by visual observation with RBOSL appearing cloudy and darker than RBO, which had a more transparent appearance. The darker cloudy color of RBOSL was because of changes that occurred during short-path distillation possibly due to sample carry over from the short-path distillation apparatus evaporation assembly.

Volatile Compounds. Table 4 shows the volatile compounds analyzed by GC-MS from SPME at 60 °C. Only 3 volatile

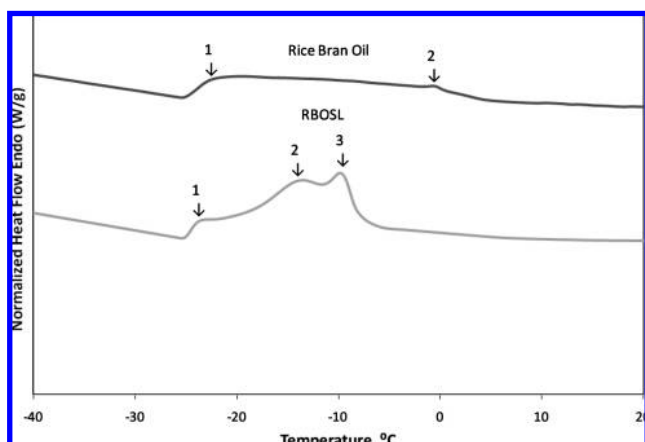
Table 4. Volatile Compounds in Rice Bran Oil and Rice Bran Oil Structured Lipid (RBOSL)

volatile compound	GC-MS peak areas	
	rice bran oil	RBOSL
2,4-decadienal	2,090,922	697,908
1-docosanol	1,366,411	
<i>n</i> -hexadecanoic acid	3,399,921	1,832,563
2-decenal		372,522
2-dodecen-1-al		405,708
octanoic acid		5,080,685

Table 5. DSC Melting Properties of Rice Bran Oil and Rice Bran Oil Structured Lipid (RBOSL)^a

property	rice bran oil	RBOSL
melting onset (°C)	-24.7 ± 0.7A	-27.1 ± 1.2A
melting end (°C)	1.8 ± 0.2A	-6.2 ± 0.8B
enthalpy ΔH (J/g)	59.6 ± 0.4A	81.7 ± 0.1B

^a Mean ± SD, *n* = 2; means with the same letter in the same row are not significantly different (*P* ≤ 0.05).

**Figure 1.** DSC thermograms of rice bran oil and rice bran oil structured lipid (RBOSL).

compounds were detected in RBO. RBO and RBOSL both contained 2,4-decadienal and *n*-hexadecanoic acid. 2,4-Decadienal and 2-decenal (detected in RBOSL) have been found in the volatile compounds detected in rice in previous studies (18, 19), and the odor of these compounds was described as fatty (19). Octanoic acid (caprylic acid) was the predominant fatty acid detected in RBOSL. This was expected because of the incorporation of caprylic acid, which is volatile. Possible reasons that so few volatile compounds were detected include the use of refined, bleached, and deodorized RBO and the use of short-path distillation to purify RBOSL, which removed more volatile compounds.

DSC Melting Profiles. Table 5 lists melting onset and end temperatures as well as ΔH values (enthalpy) for melting curves of RBO and RBOSL. The difference in melting onset temperatures for RBO and RBOSL were not significant (*P* ≥ 0.05). The melting end point temperatures, however, were significantly different from those of RBOSL showing a significantly lower melting end point temperature than RBO. The enthalpy value for RBOSL (81.7 ± 0.1 J/g) was significantly higher than that for RBO (59.6 ± 0.4 J/g) because of the stacked linear structure of the saturated fatty acid, caprylic acid, incorporated into RBOSL. Saturated fatty acids have close molecular interactions and stacked linear structures, and require more energy to melt, whereas unsaturated fatty acids have a bent structure that has

weaker molecular interactions and require less energy to melt (20). Figure 1 shows the melting curves of RBO and RBOSL. The more diverse mixture of fatty acids in the RBO TAG produces several broad melting peaks overlapping one another. The peak for RBOSL is sharper because it predominantly contains caprylic acid and has a less diverse TAG mixture.

Chemical and physical characteristics of a SL containing rice bran oil and caprylic acid were determined in this study. The differences in the chemical and physical properties of RBOSL were significantly different from those of RBO in most assays performed. RBOSL, which is liquid at room temperature, can potentially be used in butter and margarine blends, frying and baking applications, and in beverages. This characterization study can aid in determining future food applications of RBOSL.

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