OXIDATIVE STABILITY, CHARACTERIZATION AND FOOD APPLICATIONS OF A RICE BRAN OIL-BASED STRUCTURED LIPID

by

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ABSTRACT

Rice bran oil structured lipid (RBOSL) was synthesized from rice bran oil (RBO) and caprylic acid (a medium chain fatty acid) by Lipozyme RM IM lipase-catalyzed acidolysis in a continuous packed-bed reactor. The vitamin E content of RBOSL was significantly lower than RBO while the γ-oryzanol concentration of RBOSL was not significantly different from RBO ($P > 0.05$). The oxidative stability of RBOSL was significantly lower than RBO ($P \leq 0.05$). Natural and synthetic antioxidants were evaluated for their ability to increase the oxidative stability of RBOSL. The natural antioxidant carnosic acid from rosemary extract was as effective as the synthetic antioxidant TBHQ in increasing the oxidative stability of RBOSL.

RBOSL contained 32.1 mol% caprylic acid which was primarily at the *sn*-1,3 positions. Saponification value, iodine value, and viscosity were significantly different for RBO and RBOSL ($P > 0.05$). Free fatty acid content and smoke point were not significantly different for RBO and RBOSL ($P > 0.05$). Melting onset temperatures were not significantly different while endpoint and melting enthalpies were significantly different ($P \leq 0.05$).

Sweet potato chips (SPC) were fried separately in RBO and RBOSL and energy bars (EB) were formulated with RBO or RBOSL. Triangle test (TT) results for SPC showed no

significant difference in SPC fried in RBO or RBOSL and TT for EB showed a significant difference in RBO and RBOSL formulations ($P \leq 0.05$). Willingness to purchase consumer panel (5 point scale) results revealed that the most frequent response was probably would buy for SPC and EB prepared with RBOSL.

Shortening blends were formulated with RBO or RBOSL and palm stearin (PS). The caprylic acid content of shortening blends containing RBOSL and palm stearin ranged from 9.9-22.1 mol%. RBOSL blended with PS was comparable to RBO blended with PS in producing shortening with similar fatty acid profiles, solid fat content, melting and crystallization properties and crystal morphologies similar to commercial shortenings. RBOSL blended with PS can provide a healthier alternative to vegetable oils currently blended with PS to produce *trans*-free shortening.

INDEX WORDS: **A**ntioxidant, Biological catalyst, Caprylic acid, Continuous packed-bed bioreactor, Energy bar, Frying, Lipase catalyzed acidolysis, Lipozyme RM IM, Oxidative stability, Rice bran oil, Structured lipids, Sweet potato chips, Triangle test

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DEDICATION

Dedicated to my grandparents, parents, husband and children

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CHAPTER 1

INTRODUCTION

Akoh and Kim (2008) defined structured lipids (SL) as triacylglycerols (TAGs) that have been modified by 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. SL components include short chain fatty acids (SCFA), medium chain fatty acids (MCFA), and long chain fatty acids (LCFA). SCFA are lower in calories and more rapidly absorbed than MCFA or LCFA. LCFA 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. They 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 by athletes with increased energy requirements (Kennedy 1991; Megremis 1991). Previous animal and human studies have shown that the composition of TAGs containing MCFA result in increased energy expenditure and decreased weight gain (St-Onge and Jones 2002, St-Onge and others, 2003). 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 (Akoh and Kim, 2008).

Specific fatty acids can serve as acyl donors and can be esterified 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 (Jandacek, 1987).

SLs are produced enzymatically from transesterification and direct esterification reactions. In direct esterification reactions, free fatty acids are reacted with glycerol. Transesterification for SL synthesis for this study was an acidolysis reaction (a reaction between an ester and a free acid). Enzymatic esterification offers distinct advantages of positional specificity and milder reaction conditions compared to chemical esterification (Akoh, 1995; Akoh and Kim, 2008).

Lipase-catalyzed modification of TAGs can result in changes in the oxidative stability and chemical and physical characteristics of the end product different from the starting TAGs. Determination of oxidative stability and characterization of enzymatically modified TAG is necessary to improve oxidative stability and determine potential nutraceutical and food applications. Rice bran oil structured lipid (RBOSL) which is liquid at room temperature can potentially be used in frying and baking applications and in shortening blends.

OBJECTIVES

The specific research objectives were:

1. To prepare a rice bran structured lipid and determine the extent to which natural antioxidants affect the oxidative stability of rice bran oil structured lipid compared to synthetic antioxidants

2. To determine the chemical and physical characteristics of a rice bran oil structured lipid compared to the unmodified rice bran oil

3. To use a rice bran oil structured lipid for frying sweet potato chips and in an energy bar that will be acceptable to a sensory panel

4. To prepare shortening containing different ratios of palm stearin and rice bran oil structured lipid and to determine how the physical characteristics compare with commercial shortenings and blends of rice bran oil and palm stearin

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CHAPTER 2

LITERATURE REVIEW

RICE BRAN OIL

Rice bran oil is a by-product of rice milling and is consumed widely in Asia (Sharkar and Bhattacharuua, 1991). Rice bran oil for use in foods has been commercially produced in the United States since 1994. Rice bran oil has a very appealing nut-like flavor, high oxidative stability, and is well suited for frying (McCaskill and Zhang, 1999). The nutraceutical potential of tocotrienol, sterol, and γ-oryzanol in rice bran oil contributes to rising interest in rice bran oil in U.S. markets.

Gamma-oryzanol, which is a mixture of ferulic acid esters of sterols (campesterol, stigmasterol, and β-sitosterol) and terpene alcohols (cycloartanol, cycloartenol, 24-methylenecycloartanol and cyclobranol) is found on RBO and of special interest to researchers because of the health promoting properties and commercial potential offered by these bioactive compounds (Lerma-Garcia and others, 2009). γ-Oryzanol has been found to lower plasma cholesterol (Rukmini and Rughuram, 1991), reduce cholesterol absorption (Seetharamaiah and Chadrasekhara 1990) and inhibit platelet aggregation (Seetharamaiah and others, 1990). Rice bran oil consists of 38% oleic acid, 34% linoleic acid, 1% myristic acid, 22% palmitic acid, 3% stearic acid and 2% linolenic acid (Rukmini and Raghuram, 1991).

Jennings and Akoh (2000b) successfully incorporated capric acid (C10:0) into rice bran oil. This study determined the effects of incubation time, substrate mole ratio, enzyme load and water addition in a small scale study. This is an area of potential research because of the health benefits of rice bran oil would be combined with the health benefits of medium chain fatty acids (MCFA) in a rice bran oil structured lipid used in a food application.

STRUCTURED LIPIDS

Structured lipids (SLs) are produced by modifying triacylglycerols (TAGs) from their natural state to incorporate new fatty acids or to change the position of fatty acids or the fatty acid profile (Akoh and Kim, 2008). TAGs are modified to produce SL from their natural state to change their nutritional and functional properties for medical and food applications. Specific fatty acids can be targeted at specific positions to produce SL with desired chemical and physical properties.

Some examples of commercially available structured lipids containing medium chain fatty acids include Betapol™ (Loders Croklaan Company, Holland), which has a fatty acid profile similar to human milk and Bohenin which consists of behenic and oleic acids (Fuji Vegetable Oil Inc., GA). Another example is Caprenin (Proctor and Gamble, OH) which consists of long and short chain fatty acids and Salatrim (Coulter Food science, NY) which consists of short and long chain fatty acids (Akoh, 2002).

STRUCTURED LIPID COMPONENTS

Short Chain Fatty Acids

Short chain fatty acids (SCFAs) with 2 to 6 carbon fatty acids are also referred to as volatile fatty acids. They are the end products of microbial digestion of carbohydrates and occur throughout the digestive tract of mammals (Wolin, 1981) and are important to ruminant metabolism (Kristensen and Harman, 2006). The three major straight chain SCFAs are acetic, propionic, and butyric acids (Leng, 1970). Humans ingest SCFA during the consumption of bovine milk, which contains 5-10% butyric acid and 3-5% caproic acid (Brekenridge and Kuksis, 1967; Garton, 1963). SCFAs are used in the synthesis of reduced-calorie structured lipids because they contain fewer calories than LCFA or MCFA. SCFA are also more rapidly absorbed

and more soluble in water because of their smaller molecular size and chain length (Akoh and Kim, 2008). SCFA have been found to stimulate leptin production which in turn regulates physiological functions such as metabolic rate and immune function (Xiong and others, 2004).

Medium Chain Fatty Acids

Coconut oil and palm kernel oil are the major sources of MCFA which usually contain 6-12 carbons. MCFA are used to treat medical conditions such as fat absorption abnormalities in newborn infants and in patients with cystic fibrosis (Kennedy, 1991). Medium chain triacylglycerols (MCT) also have been used by athletes requiring high energy diets (Megremis, 1991). Composition of TAGs containing MCFA increases energy expenditure and decreases weight gain in animal and human studies (St-Onge and Jones 2002, St-Onge and others, 2003). MCFA are metabolized through the portal system, whereas long chain fatty acids are metabolized through the lymphatic system. MCFA are therefore quickly absorbed by the body and not stored as fat (Babayan, 1988). MCFA alone do not supply essential fatty acids (EFA) and can be toxic (Akoh, 1995). Therefore, MCFA are used in structured lipids that contain EFA on the glycerol backbone. MCFA often are targeted at the 1- and 3- positions and EFA at the 2-position of TAGs during SL synthesis. Fatty acids at the 1- and 3- positions are hydrolyzed by pancreatic lipase, and fatty acids at the 2-position are more readily absorbed than 1- and 3-position fatty acids (Haumann, 1997). Two-position fatty acids pass through the intestinal wall and are incorporated into the chylomicrons (Nettleton, 1995).

The properties that contribute to more efficient metabolism of MCFA are lower melting point, higher solubility in water and biological fluids, and smaller molecular size as compared to long chain fatty acids (Bach and Babayan, 1982). MCTs are effective in obesity control, lowering serum cholesterol and providing quick concentrated energy as well as providing a

nutritional source for the growth and physiological development of newborn infants (Babayan, 1988).

Essential Fatty Acids

Essential fatty acids cannot be synthesized by the body and must be supplied by the diet (Min and Crawford, 2004). Examples of EFA include linoleic acid (18:2*n*-6), alpha-linolenic acid (18:3*n*-3), and arachidonic acid (20:4*n*-6). These fatty acids are also considered long chain fatty acids (LCFA) and include *n*-3 and *n*-6 fatty acids. Linolenic acid is converted to gamma-linolenic acid, arachidonic acid, and alpha-linolenic acid through a series of carbon chain elongation and desaturation steps by enzymes called elongases and desaturases (Min and Crawford, 2004). Deficiencies of these fatty acids lead to the loss of excessive amounts of water through the skin and disturbances in growth and hormonal balance (Newton, 1996). Arachidonic acid is a precursor to prostanoids, thromboxane, and leukotrienes, which are known as eicosanoids. These biochemicals play a major role in immune function and platelet aggregation (Kinsella, 1988).

LIPASES

Plants, animals, bacteria, molds, and yeasts are sources of lipases which are also known as triacylglycerol ester hydrolases (EC 3.1.1.3). Lipases from microbial sources are more widely studied than other lipases because they are more readily available, being easy to isolate, and very stable, and because they can distinguish between enantiomers of chiral molecules. Lipases act at the oil water-interface and therefore do not strictly follow Michaelis Menton kinetics (Villeneuve and Foglia, 1997). Lipases have three-dimensional structures and catalyze the hydrolysis of TAG to yield monoacylglycerol (MAG), diacylglycerol (DAG), glycerol, and free fatty acids (FFA). The active site of lipases is buried under a short stretch of helix or hydrophobic lid.

During activation at the oil-water interface, the lid opens and goes into the lipid phase and exposes the substrate to the active site (Weete and others, 2008).

Lipases catalyze ester hydrolysis, direct esterification and transesterification reactions. Acidolysis, alcoholysis and interesterification are types of transesterification reactions. Acidolysis is the exchange of acyl groups between an acid and an ester; alcoholysis is acyl group exchange between an alcohol and an ester and interesterification is the exchange of two acyl groups (Willis and Marangoni, 2008).

Lipases are often immobilized to increase cost effectiveness and reusability, shorten reaction times, and control product formation and to more easily separate reactants from products (Willis and Marangoni, 2008). Immobilization is the conversion of enzymes from a water-soluble, mobile state to a water-insoluble, immobile solid state by the attachment of the enzyme to a solid support material. Adsorption can occur through ion exchange, hydrophobic, hydrophilic, and van der Waals interactions (Mustranta and others, 1993). Examples of hydrophobic support media used to immobilize enzymes include organic supports such as polyethylene, polypropylene, styrene, and acrylic polymers. Lipases generally retain the highest degree of activity when immobilized on hydrophobic supports (Malcata and others, 1990). This higher activity is ascribed to increased amounts of hydrophobic substrate at the interface (Malcata and others, 1990; Malcata and others, 1992). Among hydrophilic supports are Duolite, Celite, silica gel, activated carbon, clay, and Sepharose (Malcata and others, 1990).

Lipozyme RM IM is an immobilized lipase that catalyzes esterification and interesterification reactions. Specific interesterification reactions involve the *sn* 1 and 3 positions of triacylglycerols. Lipozyme RM IM is derived from *Mucor miehei*. The gene coding from the lipase has been transferred to *Aspergillus oryzae.* This enzyme is granular with a particle size

that ranges from 0.2-0.6 mm with a bulk density of 350-450 kg/m^3. Lipozyme RM IM is immobilized on a macroporpus anion exchange phenolic resin which is bound by adsorption without the use of crosslinking agents with an activity of 5-6 BAUN/g (Novo Nordisk, 1992).

PRODUCTION OF STRUCTURED LIPIDS BY ACIDOLYSIS

Acidolysis can be used to incorporate different fatty acids into TAGs producing SLs. The incorporation of free acids of the *n*-3 PUFAs, EPA and DHA into vegetable oils to increase their nutritional value is one example (Willis and Marangoni, 2008). Another example is cocoa butter substitutes being produced by acidolysis. In this process, 1,3-dipalmitoyl-oleoyl-glycerol from palm oil is esterified with stearic acid (Macrae, 1983; Macrae and Hammond, 1985). Other examples include the enzymatic modification of sesame oil (Jennings and Akoh, 2000a), rice bran oil (Jennings and Akoh, 2000b) and fish oils with capric acid (Jennings and Akoh, 1999; Jennings and Akoh 2001). Fomuso and others (2002) and Kim and Akoh (2006) enzymatically modified olive oil and sesame oil respectively with caprylic acid. The production of milk fat substitutes for the replacement of milk fat in baby foods by the acidolysis of palm oil top fraction, which is high in 2-palmitoyl glycerides with unsaturated fatty acids so that it more closely resembles human milk, is yet another example (King and Padley, 1990; Akoh, 2002).

ENZYMATIC INTERESTERIFICATION REACTORS

Fixed-bed reactors are a type of continuous flow reactor for enzymatic interesterification reactions. Fixed-bed reactors are well suited for large scale production and are efficient to operate and cost effective. In addition, these reactors provide a large surface area per unit volume for the enzymatic interesterification reaction to occur. Fixed-bed reactors consist of a pump to keep a constant flow rate through the system and a water-jacketed outer column to allow for constant temperature control. An immobilized enzyme is packed in the inner column and the

substrates are fed through the column, and the products exits from an opening on the opposite end of the column (Akoh and Kim, 2008).

Stirred-batch reactors are also commonly used in lipase catalyzed interesterification reactions because they are simple and economical. Reactants and products are added and removed before and after the reactions. The amount of substrate is reduced as the reaction proceeds, which decreases reaction rate. The stirred-batch reactor consists of a vessel with an agitator to mix the substrate and enzyme. Temperature is controlled by water circulated between the outer and inner wall of the vessel. Continuous stirred-tank reactors consist of an agitated tank with reactant and products are added and removed at the same rate. This type of reactor combines elements of the fixed bed and batch reactors (Akoh and Kim, 2008).

Another type of enzymatic interesterification reactors is a membrane reactor which consists of a two-phase system with the interface at the membrane. The membranes provide a large reaction surface area per unit volume. This type of reactor also results in reduced pressure drops and fluid channeling (Akoh and Kim, 2008).

PURIFICATION OF STRUCTURED LIPIDS

Distillation is a common thermal-separation method. Short-path distillation is used to separate heat sensitive high boiling point materials with very low pressures (1.0 - 0.001 mbar) which decrease the temperature necessary for evaporation. In short-path distillation evaporation occurs on a heated wiped film and is caused by the pressure drop between the place of vaporization and the vacuum system. The short distance from the evaporator surface to the condenser allows the pressure drop (Xu, 2005). Short-path distillation is used for the purification of SLs by removing free fatty acids which are vaporized and then condensed and removed as

waste. Other methods used to purify SLs include thin layer chromatography and alkaline deacidification.

PROPERTIES OF STRUCTURED LIPIDS

Enzymatic modification of TAGs results in changes in the physical and chemical properties of the resulting SLs. Kim and Akoh (2006) enzymatically modified sesame oil to contain caprylic acid and 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 that of unmodified sesame oil (Kim and Akoh, 2006). Vu and others (2008) produced a SL enzymatically produced with Lipozyme RM IM as biocatalyst from corn oil, capric acid and conjugated linoleic acid and found that the iodine value was lower in the SL while the saponification value was higher in the SL when compared to unmodified corn oil.

Oxidative Stability

Several studies have shown that the oxidative stability of SL decreases after downstream processing because of the removal of natural antioxidants such as toocopherols (Yankah and Akoh, 2000; Akoh and Moussata, 2001; Hamam & Shahidi, 2006). Health concerns over synthetic antioxidants such as tertiary butyl hydroquinone (TBHQ), which is potentially carcinogenic, has led to efforts to replace synthetic antioxidants with natural antioxidants (Shahidi, 2000). Lee and others (2004) demonstrated that 300 ppm rosemary extract (as compared to 100 and 200 ppm rosemary extract) containing 25% carnosic acid and 4% carnisol was most effective at increasing the oxidative stability of a structured lipid produced from the lipase catalyzed esterification of safflower oil and conjugated linoleic acid. Determining the oxidative stability of a SL is necessary before use in a food application.

Fatty Acid Profile

The fatty acid profiles of fats and oils from various sources as well as fats and oils modified to produce SL are unique and also determine properties such as solid fat content, melting and crystallization behavior and crystal structure and functionality in foods. The fatty acid profile can also determine the health effects of lipids. Some saturated fatty acids such as lauric, myristic and palmitic acids contribute to coronary artery disease (Mensink and Plat, 2008). Monounsaturated and polyunsaturated fatty acids such as oleic, linoleic, alpha- and gamma- linolenic, eicosapentaenoic and docohexaenoicsanoic acids have disease preventative and health promoting effects (Klurfeld, 2008; Mensink and Plat, 2008).

Gas chromatography (GC) is frequently used to determine lipid fatty acid profiles. For GC analysis volatile compounds are injected into a column containing a liquid absorbent supported on an inert solid. Separation of compounds in the volatile mixture occurs because of differences in partition coefficients of components carried through the column by an inert gas which is often helium. A flame ionization detector is commonly used for GC analysis which burns the material in a hydrogen flame producing electrons and ions. These electrons and ions move to the anode which produces a small current which is modified to produce an electrical current directly proportional to the amount of material present. Low molecular weight, volatile, nonpolar derivaties such as fatty acid methyl esters must be produced from the original lipids before GC analysis can occur. This is often by a reaction in which the TAG is displaced by an alcohol in an acid (Cserháti and Forgács, 1999).

Solid Fat Content

Determination of solid fat content (SFC) allows a description of the amounts of solid fat crystals in relation to the amount of liquid oil which gives a measure of plasticity (Johnson,

2008). A fat plastic range is the temperature range over which a fat is neither too hard or too soft or still moldable without being solid or liquid. SFC determines spreadability, sensory properties, appearance and packing properties (Ming and others, 1999, Noor Lida and others, 2002, Norizzah and others, 2004, Khatoon and Reddy, 2005, Farmani and others, 2006). Pulsed nuclear magnetic resonance spectroscopy is used to determine SFC by measuring the percent of solid fat over a temperature range usually reported from 10 to 37.8 °C (Johnson, 2008). Plasticity is an important functional property of margarine, bakery shortenings and confectionary fats.

Melting and Crystallization Profiles

Differential scanning calorimetry (DSC) determines endothermic and exothermic processes that help describe physical and chemical characteristics by measuring the effect of changing temperature on a sample. Melting and crystallization properties of fats and changes in enthalpy can be determined with DSC. Solid fats with a greater degree of hydrogenation melt at higher temperatures than liquid fats which have a lower degree of hydrogenation. The close molecular interactions and stacked linear structures of saturated fatty acids require more energy to melt.

Unsaturated fatty acids have a bent structure that has weaker molecular interactions and require less energy to melt (Ophardt, 2003). A more diverse fatty acid profile produces broad melting peaks which may overlap whereas a less diverse fatty acid profile will produce sharper peaks (Humphrey, 2003).

Crystal Properties

The 3 major polymorphic forms of fats consist of α, β and β'. These forms are also referred to as hexagonal, orthorhombic, and triclinic respectively. β' crystals, which have a

smaller thinner structure are more desirable because of their smoother mouthfeel. β' crystals can also hold more air and liquid component than larger β crystals which are more stable and have a higher melting point and a grainy texture. In comparison the α crystal form has the lowest melting point and is the least stable of the polymorphs (Lawler and Dimick, 2008).

X-ray diffraction is used to determine crystal polymorphic forms. Crystal structures are determined by diffraction patterns produced when the crystals are hit by an electron beam and produce a pattern based on electron density. α Polymorphs show d-spacings at 0.415 nm, β polymorphs at 0.420 and 0.380 nm, and β' polymorphs at 0.460, 0.385 and 0.370 nm (Lawler and Dimick, 2008).

TRANS FATTY ACIDS

The consumption of *trans* fatty acids (TFA) has been found to have negative health effects (Willet and others, 1993, Kholsa and Hayes, 1996, Pietinen and others, 1997, Mozaffarian and others, 2006). TFA are produced from the partial hydrogenation of vegetable oils. Other natural sources of TFA include the digestive tract of ruminants as well as dairy products. Partially hydrogenated fats are found in shortening, margarine, frying oils, fast food and baked goods. TFA increase LDL cholesterol levels and decrease HDL cholesterol levels which increases total plasma cholesterol levels therefore increasing the risk of cardiovascular disease (Mensink and Plat, 2008). The health risks associated with TFA have led to efforts to eliminate or significantly decrease the TFA content of many foods.

PALM OIL

Palm oil is high in palmitic, oleic, and linoleic acids and has a higher polyunsaturated fat content than coconut and palm kernel oils. Palm oil is extracted from the mesocarp of the oil palm (*Elaeis guineensis*) fruit, is economical to produce and can be fractionated into liquid

fraction (palm olein) and solid fraction (palm stearin). The color of palm oil ranges from yellow to red depending on the carotenoid content. Refining usually destroys most carotenoids and produces a light yellow color. The high oxidative stability of palm oil is due to its high tocopherol content. The carotenoids and tocopherols present in palm oil protect against certain cancers and lower serum cholesterol respectively (Edem, 2002). Palm oil does not promote atherosclerosis and arterial thrombosis although it contains 50% saturated fatty acids. The solid fat content of palm oil gives consistency without hydrogenation and can be used in food products with variable plastic ranges (Edem, 2002).

SHORTENING

Shortening provides desirable textural properties by lubricating, weakening, or shortening food components. Shortening used in frying allows uniform heat transfer and forms a moisture barrier. Other desirable functions of shortening in food include, imparting tenderness and mouthfeel, providing structural integrity, incorporation of air, and extending shelf life (Ghotra and others, 2002). Shortening is produced from the partial hydrogentation of vegetable oils. The hydrogentation process produces *trans* fatty acids which have negative health effects. Fractionation and blending of palm oil with other vegetable oils has resulted in *trans* free shortenings (Jeyarani and others, 2003).

ENERGY BARS

Energy bars are also known as nutrition bars, sports nutrition bars, granola bars and meal replacement bars were first created for athletes and body builders (Painter and Prisecaru, 2002). Although energy bars are convenient, they are believed to be no substitute for a healthy diet and proper nutrition. Energy bars offer some advantages over candy because they are lower in fat and sugars and higher in fiber. Bars that provide real food such as whole grains and dried fruits are

recommended over others (Hurley and Leibman, 2000). Foods formulated with higher carbohydrate concentrations cause more extreme fluctuations in blood sugar and can lead to insulin resistance, diabetes and weight gain. Foods formulated with added protein and fat result in more gradual increases in blood sugar (Painter and Prisecaru, 2002). Between 2001 & 2004, sales of energy bars sales were expected to increase by 10% with 1 billion in new sales added each year (Nutrition Business International 2000 & 2001, cited in Painter and Prisecaru, 2002).

FRYING

Golden brown color, crisp texture and fried food flavor are desirable sensory characteristics of fried foods. During deep fat frying food is cooked via heat transfer from the oil to the food. Frying also dries food due to mass transfer, with both processes occurring simultaneously. In addition, among the physical and chemical changes that occur are aeration, oxidation, absorption, vaporization, hydrolysis, and polymerization. These chemical reactions and their reaction products contribute to distinctive fried flavor and the type of oil, frying conditions and degradation products affect flavor quality (Warner, 2008).

Rice bran oil is a very good frying oil that imparts a pleasant flavor to fried food. Cottonseed oil, which is high in linoleic acid produces higher intensity of flavor in potato chips and french fried potatoes than oils with low linoleic acid. In addition, the low linolenic acid content of rice bran oil allows good storage stability and fry life (McCaskill and Zhang, 1999). A study by Valsalan and others (2004) assessed rice bran oils as a cooking medium and found it to be generally well accepted.

Negishi and others (2003) determined the relationship between amount of frying oil foaming and molecular weight of oils containing MCFAs. They found that some canola oil

containing MCFAs (either physical mixtures or esterified) had low foaming properties and were suitable for frying.

SWEET POTATO CHIPS

Sweet potatoes are high in nutritional value and ranked seventh as the world's most important food crop (Kays, 2006). Sweet potatoes provide 262.2% daily value (DV) of vitamin A (in the form of beta carotene), 12.6% DV of fiber, 28.4% DV of vitamin C, and 8.1% DV of iron (www.whfoods.org). Although a high yield crop that can be grown in a wide variety of areas world wide, production has remained unchanged over the last 40 years and has declined in some countries. The flavor of sweet potatoes is believed to be a factor in consumption. Processed sweet potato products are a possible means of increasing sweet potato consumption. Examples of sweet potato products that have been developed include baby food, breads, breakfast cereals, cakes, candy and chips among others (Kays, 2006).

Sweet potatoes have been marketed on small scale in the U.S. in part because current varieties have strong sweet potato flavor. Some varieties of sweet potatoes have been developed that are higher in starch, lower in sweetness and moisture than conventional U.S. sweet potatoes. These varieties produce a more desirable flavor and texture when made into chips (Becker, 2001).

Singh and others (2003) used response surface methodology to develop models to predict the product quality of sweet potato chips. Moisture loss, oil uptake, crispness, color, flavor, and texture sensory attributes were used to predict sweet potato chip product quality. Optimum conditions determined were moisture loss at 11.65% on wet basis, minimum oil uptake 2.57%, frying temperature 174.7 °C and crispness 794.37 g. Akpapunam and Abiante (1991) determined that a moisture content of 16.52% (after dehydration and before frying) resulted in

optimum texture of sweet potato chips as determined by a taste panel using a 5-point hedonic scale. Sulaeman and others (2003) demonstrated that deep fried carrot chips retain α and β-carotene content and vitamin A activity >82% for up to 5 months at various relative humidity conditions when packaged in partially vacuumed opaque pouches. Their work also demonstrates that most carotenoids can be retained at frying conditions.

SENSORY ANALYSIS

The triangle test is an overall difference test in which subjects are given two identical samples and one different sample and are asked to choose the sample different from the other two. The triangle test is used to determine whether product differences result from a change in ingredients, processing, packaging, or storage. Quantitative descriptive analysis (QDA) involves the use of a group of carefully chosen panelists that are trained to reliably identify product characteristics with results expressed on a graphical scale (Meilgaard and others, 2007). QDA test results are can be used to help further explain triangle test results.

Osborn and others (2003) conducted sensory analysis of a nutritional beverage containing a canola oil/caprylic acid structured lipid with a triangle test and qualitative descriptive analysis. For the triangle test, 23 out of 38 panelists were able to identify the odd sample containing canola oil/structured lipid versus the sample containing canola oil alone. QDA indicated the substituting the canola oil/caprylic acid structured lipid for unmodified canola oil enhanced the perception of sweet flavor and decreased bubble formation while the other attributes were unchanged.

Kim and others (2005) conducted sensory analysis of a butterfat-vegetable oil blend spread prepared with SL containing canola oil and caprylic acid by conducting a triangle test and QDA to determine the effect of SL on the sensory profile of the spread. QDA tests indicated that

the blend containing SL was significantly more cold spreadable than pure butter with a textural profile similar to the sample without SL. Significant differences were not found between the spread samples flavor attributes. These results suggest that other foods can be formulated with SLs containing caprylic acid without adversely affecting flavor and texture.

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CHAPTER 3

EFFECTIVENESS OF NATURAL VERSUS SYNTHETIC ANTIOXIDANTS IN A RICE BRAN OIL-BASED STRUCTURED LIPID

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ABSTRACT

Antioxidants were evaluated for their ability to improve oxidative stability index (OSI) of enzymatically prepared rice bran oil-based structured lipid (RBOSL) containing caprylic acid. RBOSL was purified by short-path distillation. Vitamin E concentration decreased significantly in RBOSL after enzymatic modification. Total γ-oryzanol concentration after modification was not significantly different. OSI of RBOSL containing rosemary extract (RE), carnosic acid (CA), tertiary butyl hydroquinone (TBHQ), ethylenediamine tetraacetic acid (EDTA), and α-tocopherol (TOC), and 50:50 (w/w) combinations in concentrations of 200, 300, 400 and 500 ppm were determined. The OSI of unmodified rice bran oil (RBO) was 12.4 ± 0.2 hr and significantly higher than RBOSL which was 11.4±0.0. Mean peroxide and *p*-anisidine values for antioxidant treatments in RBOSL with the highest OSI values were determined after incubation at 60 °C for 21 days and sampled every 3 days. Mean peroxide and *p*-anisidine values for CA and CA/RE were comparable to TBHQ.

keywords: Antioxidant, Enzymatic modification, Oxidative stability, Rice bran oil, Structured lipid

Introduction

The nutraceutical potential of tocotrienol, sterol, and γ-oryzanol in rice bran oil (RBO) as well as its flavor, oxidative stability, and frying performance contributes to the rising interest in RBO product in U.S. markets (McCaskill & Zhang, 1999). The γ-oryzanol in rice bran oil is a mixture of ferulic acid esters of sterols (campesterol, stigmasterol, and β-sitosterol) and terpene alcohols (cycloartanol, cycloartenol, 24-methylenecycloartanol and cyclobranol). RBO contains γ-oryzanol, which is a ferulic acid ester of sterols and terpene alcohols. γ-Oryzanol has been found to lower plasma cholesterol (Rukmini and Rughuram 1991), reduce cholesterol absorption (Seetharamaiah and Chadrasekhara 1990) and inhibit platelet aggregation (Seetharamaiah et al., 1990). RBO consists of approximately 38% oleic, 34% linoleic, 1% myristic, 22% palmitic, 3% stearic, and 2% linolenic acids (Rukmini and Raghuram, 1991).

The health benefits of RBO can possibly be improved by enzymatic modification which incorporates medium chain fatty acids. Jennings and Akoh (2000) successfully incorporated capric acid (C10:0) into RBO and determined the effects of incubation time, substrate mole ratio, enzyme load, and water addition in a small-scale study. The health benefits of medium chain triacylglycerols such as those containing capric and caprylic acid have been outlined by Kennedy (1991) and Megremis (1991). Additional studies involving the enzymatic modification of rice bran oil have been limited. Because of the possible combined health benefits of RBO enzymatically modified to contain a medium chain fatty acid, further studies are needed to determine the chemical and physical characteristics. These studies also should investigate the oxidative stability of SL produced in kilogram quantities to be used in a food applications.

Several studies have shown that the oxidative stability of SL decreases after downstream processing because of the removal of natural antioxidants such as tocopherols (Yankah & Akoh, 2000; Akoh & Moussata, 2001; Hamam & Shahidi, 2006). Health concerns over synthetic antioxidants such as tertiary butyl hydroquinone (TBHQ) and butylated hydroxyanisole (BHA), which are potentially carcinogenic (Peters, Rivera, Jones, Monks and Lau, 1996) have led to efforts to replace synthetic antioxidants with natural antioxidants (Shahidi, 2000). Lee, Shin, Lee & Lee, (2004) and Lee, Lee, Akoh, Chung & Kim (2006) demonstrated that rosemary extract at 100, 200 and 300 ppm was effective at increasing the oxidative stability of a structured lipid produced from the lipase-catalyzed esterification of safflower oil and conjugated linoleic acid and extra virgin olive oil and conjugated linoleic acid. Among advantages of natural antioxidants are wide acceptance by consumers and health officials, potential to be labeled as flavorings and positive affects on sensory properties (Pokorný, 2007). The oxidative stability of structured lipids should be determined and antioxidants added, if necessary, to improve oxidative stability before use in a food application.

The objectives of this study were to modify the fatty acid profile of RBO to contain caprylic acid and to compare the antioxidant capability of the synthetic antioxidant, tertiary butylhydroquinone (TBHQ), to that of natural antioxidants and antioxidant combinations added to a rice bran oil-based structured lipid. The tocopherol content and the γ-oryzanol content of rice bran oil before and after enzymatic modification were also determined.

Materials and Methods

Materials

RBO was purchased from California Rice Oil Company (Novato, CA). Caprylic acid, EDTA, α-tocopherol, and TBHQ were purchased from Sigma Chemical Co. (St. Louis, MO). Carnosic acid was purchased from A.G. Scientific (San Diego, CA). Rosemary extract was donated by Kalsec (Kalamazoo, MI). Lipozyme RM IM (immobilized lipase on a macroporous anion exchange resin) from *Rhizomucor miehei* was donated by Brenntag Mid-South, Inc. (Atlanta, GA).

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 1ml/min, 1:6 substrate mole ratio (rice bran oil: caprylic acid), and a temperature of 45 °C (Kim & Akoh, 2006). A bioreactor with a jacketed stainless steel column (47 mm x 500 mm) and a FMI Lab 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 (2002). 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 4 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 was15 °C, and the vacuum pressure was below 1 Torr (Fomuso & Akoh, 2002).

Gas chromatography

Gas liquid chromatography (GLC) was used to determine the fatty acid profiles of rice bran oil-based structured lipid and the unmodified rice bran oil. Fatty acid methyl esters were prepared as described by Jennings and Akoh (2000). The gas chromatograph was an Agilent 6890N (Wilmington, PA) equipped with an AT-225 fused-silica capillary column 30 m x 0.25 mm i.d. (Alltech, Deerfield, IL), flame-ionization detector, and operated in a 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 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 23 ml/min. The relative concentration of FAME as mol% was calculated by computer with 17:0 as internal standard.

Tocopherol analysis

RBO and RBOSL were analyzed on a normal phase HPLC system equipped with a Shimadzu LC-6A pump, a Shimadzu RF-10A spectrofluorometric detector, a Spectra series AS 100 autosampler (Thermo Separation Products Inc., San Jose, CA) and a LiChrosorb Si 60 column (25cm x 4mm, 5 µm; Hibar Fertigsaube RT, Darmstadt, Germany) equipped with a precolumn packed with Perisorb A 30-40 µm (Fertigsaube RT, Darmstadt, Germany). Tocopherols and tocotrienols were identified and quantified as described by Ye, Landon, Lee and Eitenmiller (1998). The isocratic mobile phase contained 0.9% isopropanol in hexane at a flow rate of 1.0 ml/min. The wavelengths for excitation and emission were 290 and 330 nm, respectively (Lee, Landen, Phillips & Eitenmiller, 1998).

γ-Oryzanol analysis

HPLC analysis to determine γ-oryzanol content of rice bran oil before and after modification was performed with an Agilent (Wilmington, PA) model 1100 liquid chromatograph equipped with a diode array UV-visible detector. γ-Oryzanol standards were prepared from γ-oryzanol powder purchased from TCI America (Portland, OR). The stationary phase was a Phenomenex (Torrence, CA), ODS-2, RP C_{18} column. The initial mobile phase conditions were 45% acetonitrile, 40% methanol, 5% isopropyl alcohol, and 10% aqueous acetic acid. After 4 min, the mobile phase was changed linearly to 25% acetonitrile, 70% methanol, 5% isopropyl alcohol for 10 min and held for 11 min with a total run time of 25 min. After each run, the solvent

gradient was changed linearly to 95% methanol and 5% isopropyl alcohol and held for 1 min before returning to initial mobile phase conditions. γ-Oryzanol peaks were detected at 325 nm and quantified from a standard curve (Chen & Bergman, 2005).

Determination of oxidative stability

An oxidative stability instrument (Omnion, Inc., Rockland, MA) was used to determine the oxidative stability index of RBO and RBOSL. American Oil Chemists' Society (AOCS,1998) official method Cd 12b-92 was used. The instrument temperature was set at 110 °C and the air flow rate was 2.5 ml/s. Peroxide value was determined by AOCS official method Cd 8b-90. The *p*-anisidine value was determined by AOCS official method Cd 18-90. The antioxidants tested in RBOSL were rosemary extract (RE), carnosic acid (CA), tertiary butyl hydroquinone (TBHQ), ethylenediamine tetraacetic acid (EDTA), and α-tocopherol (TOC) in concentrations of 200, 300, 400 and 500 ppm. In addition, four other separate treatments of 50:50 combinations (in equal concentrations that total 200, 300, 400 & 500 ppm) of TOC/RE, TOC/TBHQ, TOC/EDTA, RE/TBHQ, EDTA/TBHQ, CA/RE, CA/TBHQ, CA/EDTA and RE/EDTA were added to the RBOSL. The control was RBOSL without added antioxidants. The oxidative stability index was determined for each treatment. Fifty grams of RBOSL with antioxidant treatments at 300, 400 and 500 ppm originally tested with the highest OSI values were incubated in an open flask at 60 °C in an oven for 21 days (Hamilton & Rossel, 1986). Peroxide value and *p*-anisidine value were determined as described above in triplicate at days 0, 3, 6, 9, 12, 15, 18, and 21.

Statistical analysis

Statistical analysis was conducted with the SAS software package (SAS Inst., 2000). A t-test was used to determine the differences between mean OSI values of RBO and RBOSL without added antioxidant. A two-way analysis of variance (ANOVA) and mean separation tests were used to evaluate differences among mean OSI values from the various antioxidant treatments. The Ryan-Einot-Gabriel-Welch mean separation test was used to determine differences among mean OSI values. The Bonferroni mean separation test was used to determine differences between mean peroxide values and mean p-anisidine values. Statistical differences with $P \leq 0.05$ were considered significant.

Results and Discussion

Rice bran oil was enzymatically modified to contain 32.1 mol% caprylic acid (Table 3.1). Myristic, palmitic, oleic, linoleic, and linolenic acids were significantly lower for RBOSL than in unmodified RBO decreasing by 0.4, 13.3, 2.0, 10.6, 6.2 and 0.4 mol% respectively, indicating that caprylic acid replaced the other fatty acids. Total monounsaturated fatty acid content and polyunsaturated fatty acid content decreased by 10.6 and 6.6 mol%, respectively, in the RBOSL.

Tocopherols and Tocotrienols

The vitamin E content of RBOSL as compared to RBO was significantly lower by 43.4% for all tocopherol and tocotrienol homologs except δ-tocopherol which was not significantly

different (Table 3.2). δ -Tocopherol is more stable than α-tocopherol which oxidizes to tocopheryl radicals at a faster rate (Nogala-Kalucka, Korczak, Elmadfa & Wagoner, 2005). The waste from short-path distillation in this study was analyzed and found to contain α-tocopherol , γ-tocopherol and γ-tocotrienol at 2.2±.0.0, 2.5±0.1 and 7.6±0.9 mg/100 ml, respectively, indicating that significant amounts of vitamin E were lost in the waste during the short-path distillation. Kamal-Elden & Appleqvist (1996) indicated that δ-tocopherol has a higher antioxidant activity than the other tocopherol homologs. Jennings and Akoh (2000) demonstrated that the vitamin E content of the enzymatically modified rice bran oil was slightly lower than the unmodified rice bran oil. The difference in results was probably due to the difference in enzymatic modification and purification methods. In the earlier study by Jennings and Akoh (2000), enzymatically modified rice bran oil was prepared in mg quantities in a culture tube and purified by thin layer chromatography.

γ- Oryzanol

The concentration of γ-oryzanol measured by comparing the concentration of the γ-oryzanol components, cycloartenyl ferulate, 24-methylenecycloartenyl ferulate and camperseryl ferulate in RBO and RBOSL were not significantly different from each other (Table 3.3). γ-Oryzanol content of rice bran oil has been shown not to change after frying for up to 1 hr at 180 °C (Krishna, Katoon & Babylatha, 2005). The high stability of rice bran oil as a frying oil is attributed in part to its γ-oryzanol content. A study by Nyström, Achrenius, Lampi, Moreau & Piironen (2007) indicated that the γ-oryzanol component, sitosteryl ferulate, degraded at a slower

rate than α-tocopherol and that these compounds did not exert a synergistic effect as antioxidants.

Oxidative stability index

Table 3.4 shows the oxidative stability index (OSI) values (obtained at 110 °C) at different antioxidant concentrations. The OSI value of RBO without antioxidant addition (12.4 ±0.2 h) was significantly higher than the OSI value for RBOSL (11.4±0.0 h). The OSI value for EDTA/TBHQ at 500 ppm was the highest of all antioxidant treatments with an OSI value of 27.5±0.4 h. The increased mean OSI values for EDTA/TBHQ indicated a strong synergistic effect between the metal chelator EDTA and TBHQ. The antioxidants EDTA/TBHQ, CA/TBHQ, CA/RE, CA, and TBHQ were among those that showed the highest OSI values and were significantly higher than the OSI value for RBOSL without added antioxidant. Mean OSI values increased for most antioxidants as antioxidant concentrations increased.

When compared at 200 ppm (the maximum limit for the amount of TBHQ that can be added to oils in the U.S.A.), TBHQ, CA/RE and CA/TBHQ were not significantly different. Lee, Shin, Lee & Lee (2004) also found that rosemary extract reduced oxidation of a safflower oil based structured lipid. EDTA and TOC antioxidant treatments at 400 ppm were the least effective with OSI values of 9.8±0.4 h and 9.8±0.5 h respectively and were significantly below the OSI value for RBOSL without added antioxidant. The mean OSI value for α-tocopherol added to RBOSL at 400, and 500 ppm was significantly lower than that of the RBOSL without added antioxidant while at 200 and 300 ppm α-tocopherol, there was no significant difference

from RBOSL without added antioxidant. These results were possibly because α-tocopherol concentrations were above the optimum concentration of 250 ppm. At greater than 250 ppm, α-tocopherol becomes a prooxidant or may exert a prooxidant synergistic effect when combined with transition metal ions or lipid hydroperoxides (Kulas & Ackman, 2001).

TOC/TBHQ at 500 ppm was not significantly different from TBHQ at 500 ppm with mean OSI values of 20.4±0.6 h and 19.7±0.6 h, respectively. Akoh and Moussata (2001) also found that a 50:50 combination of TOC/TBHQ at 50 and 100 ppm was effective in increasing the oxidative stability of a fish oil and canola oil-based structured lipid. The 50:50 combination of the natural and synthetic antioxidants CA/TBHQ and TOC/TBHQ mean OSI values at 500 ppm were 21.4±0.1h and 20.4±0.6 h respectively, and were not significantly different. These values also were not significantly different from TBHQ at 500 ppm. Therefore, a combination of natural and synthetic antioxidants may be an alternative to totally replacing synthetic antioxidants with natural antioxidants allowing lower levels of synthetic antioxidants to be used.

The mean OSI value of the rosemary extract phenolic compound CA at 500 ppm was 19.8±0.6 h. The mean OSI values for rosemary extract (RE) at 200, 300, 400, and 500 ppm ranged from 11.4±0.4 h to 12.5±0.9 h and were not significantly different from the OSI value of 11.4±0.0 h for RBOSL without added antioxidant. These results may have been because of low concentrations of the rosemary extract phenolic compounds carnosic acid, carnisol and rosmarinic acid as well as other compounds. The 50:50 combination of CA/RE at 500 ppm had an OSI value of 19.6±0.2 h which was not significantly different from CA at 500 ppm with a mean OSI value of 19.8±0.6 h. Use of purified carnosic acid (97.4%) allowed a more controlled

amount of carnosic acid used as an antioxidant for this study. Carnosic acid is the most abundant and the most active antioxidant in rosemary extract. The amount of carnosic acid in rosemary extract varies, possibly making some rosemary extracts less effective antioxidants. A disadvantage of the use of purified carnosic acid from rosemary extract as an antioxidant treatment was high cost. Another possible disadvantage is toxicity. However, a study by Anadón, Martínez-Larrañaga, Matínez, Ares, García-Risco, Señoráns and Reglero (2008) showed that rosemary extracts consisting of predominately carnosic acid fed to rats at 2000 mg/kg produced no adverse effects. The use of a rosemary extract with 24.6% carnosic acid which have been shown to be effective in structured lipids (Lee et al., 2004 and Lee et al., 2006) rather than purified carnosic acid would be more feasible from a cost perspective.

Peroxide values and p-Anisidine values

EDTA/TBHQ, CA/TBHQ, CA/RE, CA and TBHQ antioxidant treatments of RBOSL at 300, 400 and 500 ppm were among the highest OSI values obtained at 110 °C (Table 3.4). These treatments were then incubated at 60 °C and peroxide and *p*-anisidine values were determined at 0, 3, 6, 9, 12, 15, 18, and 21 days. Figure 3.1 shows peroxide values that were significantly lower than that of RBOSL without added antioxidant. Generally, as incubation time increased, peroxide value also increased. Peroxide value which measures hydroperoxide formation, is an indicator of primary oxidative changes. Most treatments and concentrations on given sampling days yielded peroxide values that were not significantly different from RBOSL. There was no significant difference among peroxide values at different treatment concentrations which possibly indicate that antioxidant concentrations at 300 ppm or lower were sufficient to lower

peroxide value. For day 21, none of the treatments were significantly different form RBOSL without added antioxidant. Other studies (Lee et al., 2004; Lee et al., 2006) also showed lower peroxide and *p*-anisidine values for structured lipids containing safflower oil and conjugated linoleic acid or extra virgin olive oil and conjugated linoleic acid with added rosemary extract with 24.6 % carnosic acid than the control without added antioxidant. Overall, CA/RE, CA, EDTA/TBHQ, CA/TBHQ and TBHQ performed well in lowering the peroxide value to levels significantly lower than RBOSL through day 18. These findings indicate that the natural antioxidants CA and CA/RE are as effective as TBHQ in lowering peroxide values in RBOSL.

The decomposition of hydroperoxides into secondary oxidation products causes undesirable secondary flavor and odor compound formation. *p*-Anisidine value is a measure of the amount of aldehydes present, which are secondary oxidation carbonyl compounds. Figure 3.2 shows *p*-anisidine values that were significantly lower than that of RBOSL without added antioxidant. *p*-Anisidine values for day 0 for all treatments were significantly lower than RBOSL without added antioxidant. None of the *p*-anisidine values were significantly different from RBOSL without added antioxidant on day 3. *p*-Anisidine values for treatments at 500 ppm were generally lower than at other concentrations. Mean *p*-anisidine values for CA 500 ppm on days 6, 9, 12 and 15 were significantly lower than RBOSL without added antioxidant and were comparable to TBHQ at 500 ppm. The 500 ppm may be acceptable for natural antioxidants, but not for TBHQ, which can only be added up to 200 ppm. *p*-Anisidine values increased as incubation time at 60 °C increased and the largest increases in *p*-anisidine values occurred between days 3 and 9. On day 18, all *p*-anisidine values were significantly higher than RBOSL without added antioxidant indicating an increased rate of *p*-anisidine formation. On day 21, *p*-anisidine values for CA 300 ppm and TBHQ 300 ppm were comparable and significantly

lower than RBOSL and overall levels of *p*-anisidine were lower than on day 18. *p*-Anisidine values for TBHQ, CA and CA/RE on days 0, 6, 9, 12 and 15 were significantly below RBOSL without added antioxidant and results were generally in agreement with findings from peroxide value results indicating that the effectiveness of natural and synthetic antioxidants were similar.

Conclusion

Rice bran oil was enzymatically modified to contain caprylic acid. The vitamin E content was significantly lower in RBOSL than in unmodified RBO because of the removal of tocopherols during short-path distillation. Differences in γ-oryzanol concentration before and after enzymatic modification were not significant. OSI values for CA and CA/RE were not significantly different from TBHQ. Peroxide values for CA and CA/RE were significantly lower than RBOSL without added antioxidant but not statistically different from TBHQ at days 9, 15, and 18. Mean *p*-anisidine values for CA 500 ppm on days 6, 9, 12 and 15 were significantly lower than RBOSL without added antioxidant and were comparable to TBHQ 500 ppm. The natural antioxidants, CA and CA/RE, were therefore shown to be as effective as TBHQ in increasing the oxidative stability of RBOSL. The effectiveness of combinations of natural and synthetic antioxidants such as CA/TBHQ is also notable and can provide an alternative that will allow for lower amounts of synthetic antioxidants to be used. The possible carcinogenic effect of synthetic antioxidant TBHQ and consumer demand for natural healthier products make natural antioxidants more desirable. Natural antioxidants are more accepted by consumers and health officials, can be labeled as flavorings, and may have positive effects on sensory properties. Our

results indicate that natural antioxidants are viable alternatives to the synthetic antioxidant TBHQ for increasing the oxidative stability of a rice bran oil-based structured lipid.

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Table 3.1. Fatty acid profile of rice bran oil before and after modification (mol%)

Fatty Acid	Before	After
8:0	0.0±0.0	32.1±0.9
14:0	0.7±0.0	0.3±0.1
16:0	24.8±0.3	11.5±0.3
18:0	2.0±0.0	0.0±0.0
18:1*n*-9	37.6±0.1	27.0±0.6
18:2*n*-6	33.3±0.2	27.1±0.2
18:3*n*-3	1.2±0.0	0.8±0.0
20:0	0.5±0.0	0.5±0.0

Mean±SD, n=2

Table 3.2. Vitamin E content of rice bran oil (RBO) in mg/100 ml before modification and rice bran oil-based structured lipid (RBOSL) after modification

	α-T	β-T	γ-T	δ-T	α-T3	γ-T3	δ-T3	Total
RBO	9.9±1.5	0.3±0.0	8.7±0.1	1.0±0.3	2.4±0.7	36.4±0.6	0.9±0.2	59.6±0.9
RBOSL	3.3±0.5	0.0±0.0	1.1±0.1	1.6±0.7	1.0±0.3	9.2±0.3	0.0±0.0	16.1±1.0

T=tocopherol; T3=tocotrienol
Mean±SD, n=2

Table 3.3. Gamma oryzanol major components of rice bran oil (RBO) before modification and rice bran oil-based structured lipid (RBOSL) after modification (μg/ml)

	Cycloartenyl Ferulate	24-Methylenecycloartenyl Ferulate	Campesteryl Ferulate	Total
RBO	1050.0±36.0	447.0±4.8	5503.0±94.5	7000.0
RBOSL	1130.1± 1.2	601.5±3.8	5645.6±2.7	7377.2

Mean±SD, n=2

Table 3.4. Oxidative stability index (OSI) values (h) at different antioxidant concentrations

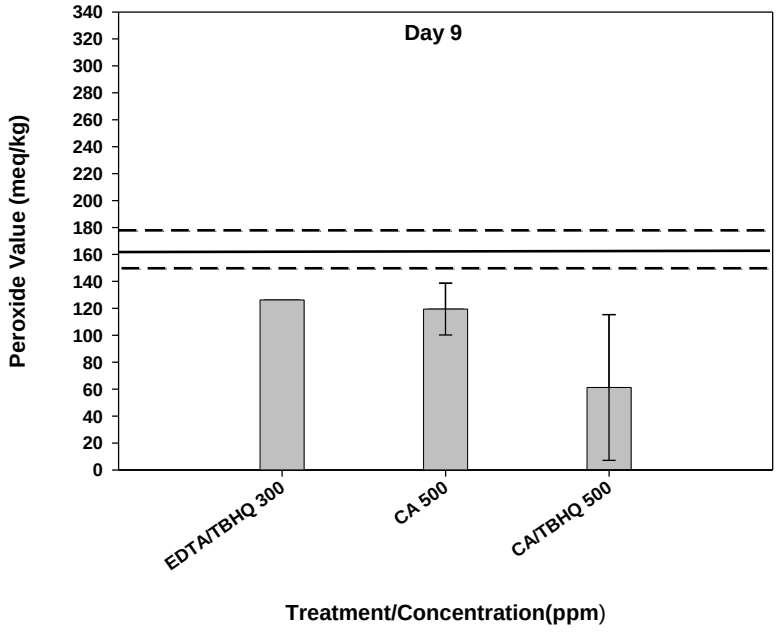
Antioxidant	Concentration (ppm)				
	0	200	300	400	500
EDTA/TBHQ	-	19.1±0.3a	22.0±0.2a	24.2±0.2a	27.5±0.4a
CA/TBHQ	-	15.9±0.4a	17.3±0.1a	18.8±0.4a	21.4±0.1a
CA/RE	-	16.1±1.1a	17.3±1.1a	18.1±0.1a	19.6±0.2a
CA	-	14.3±0.8a	18.0±0.7a	18.8±1.4a	19.8±0.6a
TBHQ	-	15.9±1.3a	16.9±0.2a	17.4±0.5a	19.7±0.6a
TOC/TBHQ	-	14.2±0.2a	16.6±0.2a	18.8±0.4a	20.4±0.6a
CA/EDTA	-	14.8±0.6a	15.2±0.4a	16.8±0.1a	17.9±0.1a
RE/TBHQ	-	13.5±1.2b	14.7±0.1a	16.1±1.6a	16.4±0.3a
CA/TOC	-	13.7±0.3a	13.1±0.1a	13.8±0.8a	15.3±0.1a
RBO	12.4±0.2	-	-	-	-
RE	-	11.6±1.1b	11.4±0.4b	12.1±0.5b	12.5±0.9b
RE/TOC	-	11.6±0.2b	11.4±0.6b	11.4±0.5b	11.9±0.5b
RBOSL	11.4±0.0	-	-	-	-
TOC/EDTA	-	10.6±0.3c	10.6±0.3c	10.5±0.4c	10.7±0.2b
RE/EDTA	-	10.0±0.1c	10.6±1.4b	11.2±1.2c	10.3±0.3c
TOC	-	10.4±0.8b	10.7±0.5b	9.8±0.5c	10.0±0.1c
EDTA	-	10.5±1.3b	10.2±0.6b	9.8±0.4c	10.0±0.6c

RBOSL= rice bran oil structured lipid without added antioxidant, RBO=rice bran oil without added antioxidant, Rosemary extract = RE, carnosic acid = CA, tertiary butyl hydroquinone = TBHQ, ethylenediaminetetraacetic acid = EDTA and α-tocopherol = TOC. a=mean significantly higher than RBOSL without added antioxidant, b= mean not significantly different from RBOSL without added antioxidant, c=mean significantly lower than RBOSL without added antioxidant.
Mean±SD, n=3

Captions to figures

Figure 3.1. Bar graphs of peroxide values significantly lower than RBOSL at 9, 15, and 18 days. Peroxide values for days 0 and 3 were equal to 0 and graphs are not shown. Peroxide values for days 6, 12 and 21were significantly higher than or not significantly different from RBOSL and are not shown. Solid lines across y axis represent mean peroxide value of RBOSL without added antioxidant. Dotted lines across y axis represent 95% upper and lower confidence intervals for RBOSL. Graphs represent Bonferroni mean separation test results. Lines on top of bars represent 95% upper and lower confidence intervals. No confidence interval lines appear where replicate values were the same. Peroxide values determined in triplicate. Rosemary extract = RE, carnosic acid = CA, tertiary butyl hydroquinone = TBHQ, and ethylenediaminetetraacetic acid = EDTA. Antioxidant concentrations are 300, 400, and 500 ppm. Treatments with two antioxidants separated by back slash are 50:50 combinations that equal 300, 400 and 500 ppm total antioxidant. Peroxide values are listed in descending order.

Figure 3.2. Bar graphs of *p*-anisidine values significantly lower than RBOSL at 0, 6, 9, 12, 15, and 21 days. Y-axis scale maximum of 100 mg/kg for day 0 different from other days maximum of 200 mg/kg to more clearly show contrast between bars and mean RBOSL and confidence interval lines. Solid lines across y axis represent mean *p*-anisidine value of RBOSL without added antioxidant. Dotted lines across y axis represent 95% upper and lower confidence intervals for RBOSL. *p*-Anisidine values for days 3 and 18 were not significantly different from or significantly higher than RBOSL, respectively, and are not shown. Graphs represent Bonferroni mean separation test results. Lines on bars represent 95% upper and lower confidence intervals. No confidence interval lines appear where replicate values were the same. *p*-Anisidine values determined in triplicate. Rosemary extract = RE, carnosic acid = CA, tertiary butyl hydroquinone = TBHQ, and ethylenediaminetetraacetic acid = EDTA. Antioxidant concentrations are 300, 400, and 500 ppm. Treatments with two antioxidants separated by back slash are 50:50 combinations that equal 300, 400 and 500 ppm total antioxidant. *p*-Anisidine values are listed in descending order.



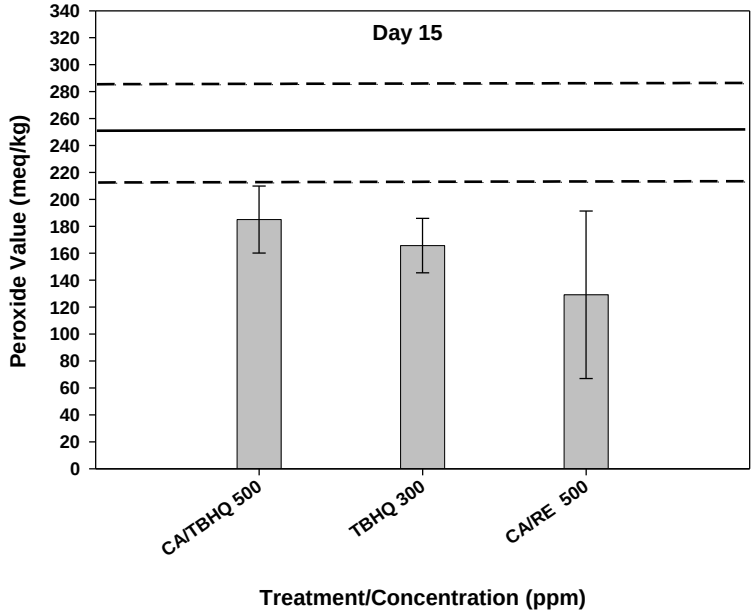


Figure 3.1., Jennings and Akoh

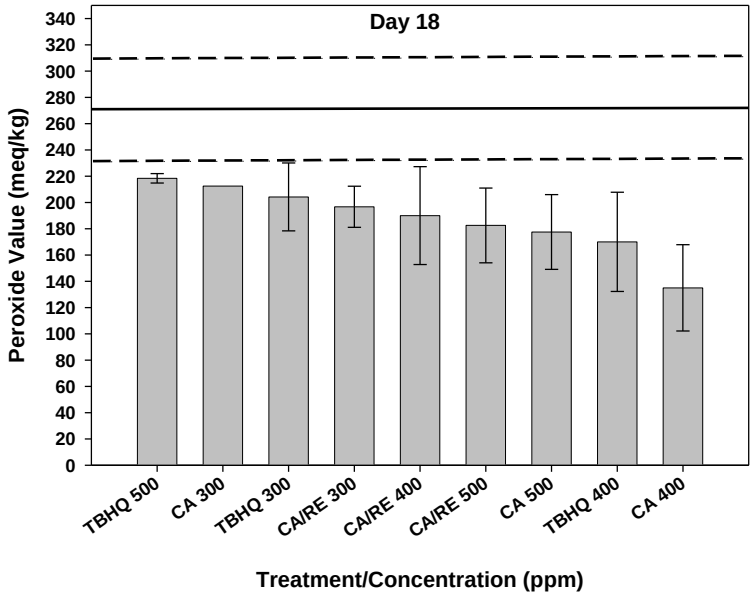
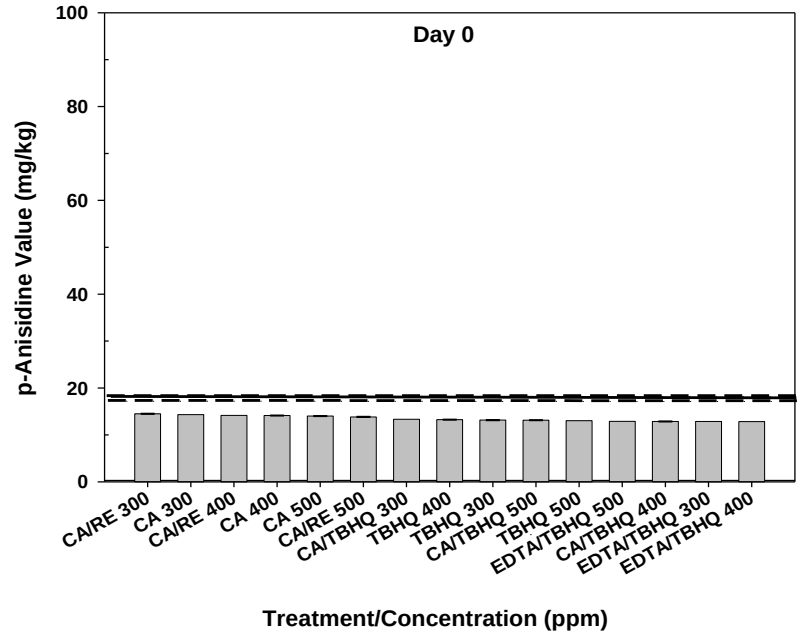


Figure 3.1., Jennings and Akoh



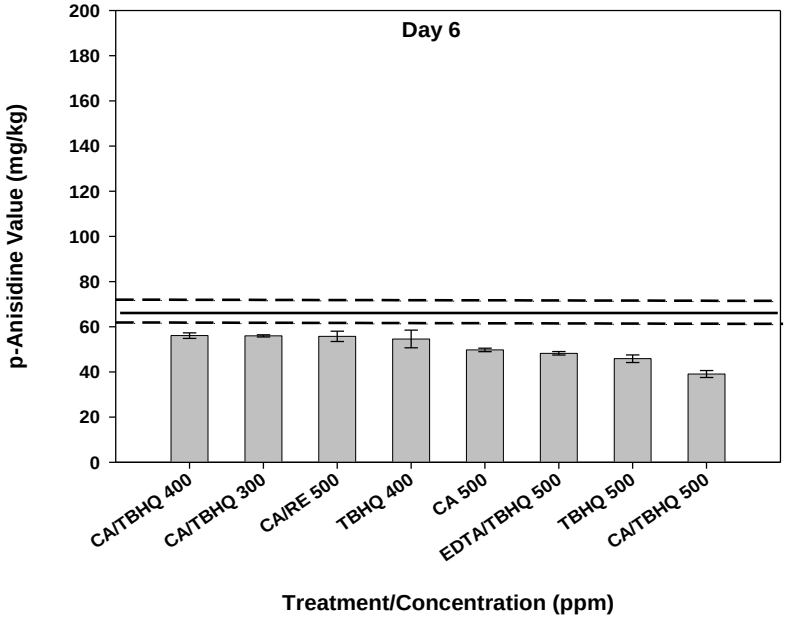
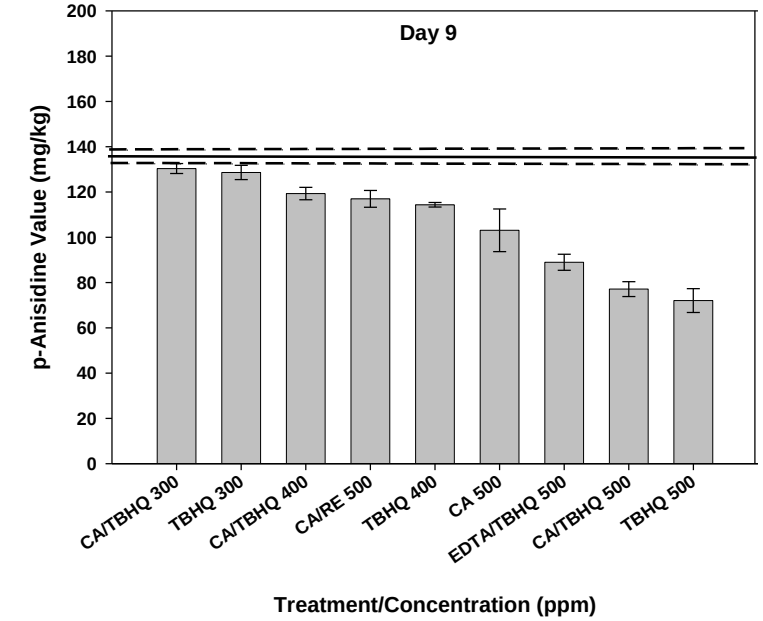


Figure 3.2, Jennings and Akoh



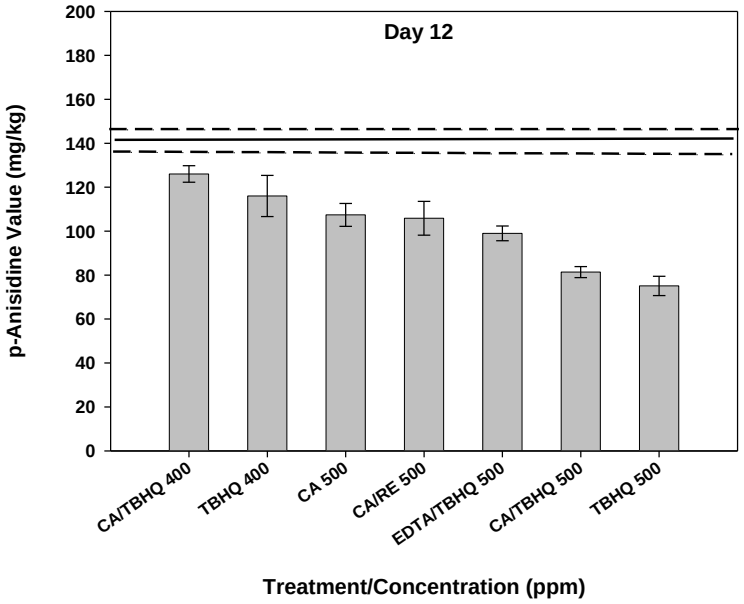


Figure 3.2., Jennings and Akoh

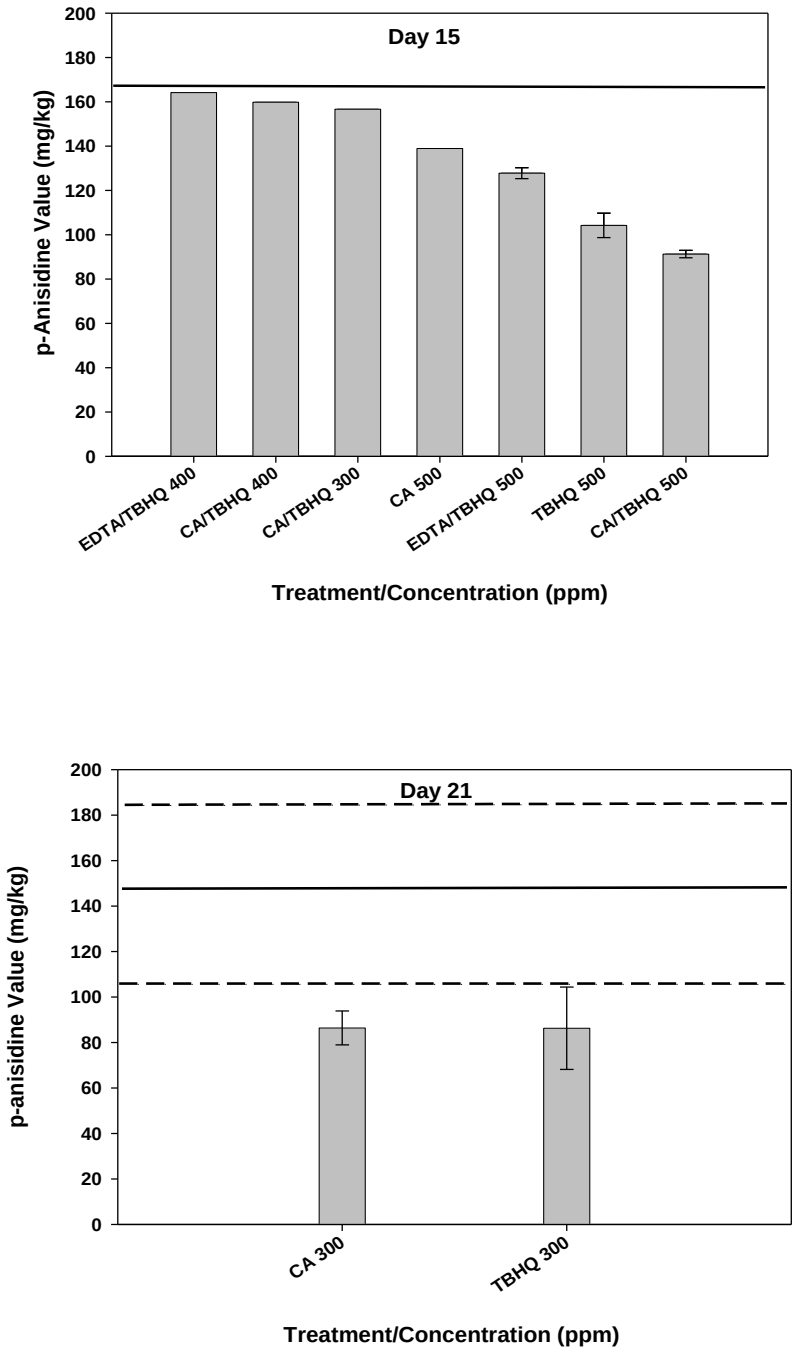


Figure 3.2., Jennings and Akoh

CHAPTER 4

CHARACTERIZATION OF A RICE BRAN OIL STRUCTURED LIPID

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ABSTRACT

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 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 endpoint 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 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). SCFAs are lower in calories and more rapidly absorbed than MCFAs or LCFAs. 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 LCFAs. 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 SLs. Therefore, the characteristic component fatty acids of SLs determine the physical properties, digestion, absorption, and metabolism of SLs. 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 that 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 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 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 macroporous anion 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 1ml/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 x 500 mm) and a FMI Lab 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 x 0.25 mm i.d. (Alltech, Deerfield, IL), flame-ionization detector, and operated in a 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 concentrations of FAMEs as mol% were 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 M Tris buffer (pH 8.0), 0.25 ml of bile salts (0.1%), 0.2 ml of $CaCl_2$ (22.0%), and 8.0 mg of purified pancreatic lipase was added to the reaction mixture (*12*). The mixture was then incubated for 3 minutes 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 *sn*-2 monoacylglycerol (MAG) standard were scraped from the TLC plate. The *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 °C, ΔH 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 °C 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 (25g) 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 1hr 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 30m x 0.25 mm x 0.25µm 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; 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 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 for GC above. Mass spectra were obtained at a MS voltage of 70Ev. 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 7th 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 at 22.8 °C with a RV Brookfield Digital Viscometer (spindle #2) (Brookfield Engineering Laboratories, Inc., Stoughton, MA). Color was measured by a Minolta CR-300 Chroma Meter (Osaka, Japan) using la Commission Internationale de l'Eclairage (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 $P \leq 0.05$ were considered significant.

RESULTS AND DISCUSSION

Positional Analysis. Unmodified rice bran oil did not contain caprylic acid (**Table 4.1**). 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 *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 (**2. 2**). The FFA content of RBOSL after enzymatic modification in the packed-bed reactor was high and 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 ($P > 0.05$) 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 saponfication value measures the alkali reactive groups in oils in mg 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 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 (**2. 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 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 2.7g/100 ml after refining, bleaching and deodorizing (*16*). A previous study (*17*) in which rice bran oil was 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 RBO (12.4±0.2 h). Another study that determined the characteristics of a SL prepared from unrefined sesame oil and caprylic acid found that the concentrations of the tocopherols, phytosterols, and lignins were not significantly different ($P > 0.05$) 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 at 22.8 °C (66.8±0.2 cP) was

significantly lower than that of RBO (78.6±0.2 cP) (Table 4.2). Caprylic acid which replaced long chain fatty acids has a lower molecular weight and density than fatty acids present in RBO and these factors 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 4.3**). 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 then 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 4.4 shows the volatile compounds analyzed by GC-MS from SPME at 60 °C. Only 3 volatile 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), has 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.

Melting Profiles. Table 4.5 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 endpoint temperatures, however, were significantly different from RBOSL showing a significantly lower melting endpoint temperature than RBO. The enthalpy value for RBOSL (81.7±0.1 J/g) was significantly higher than 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 4.1** 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 contains predominantly 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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Table 4.1. 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)

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±.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	-	-	-	-

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

Table 4.2. Chemical and Physical Properties of Rice Bran Oil and Rice Bran Oil Structured Lipid (RBOSL)

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 B	206.87±0.7 A
iodine value	92.9±0.2 A	82.2±0.6 B
viscosity (cP) (22.8 °C)	78.6±0.2 A	66.8±0.2 B

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

Table 4.3. CIE L*a*b* Color of Rice Bran Oil and Rice Bran Oil Structured Lipid (RBOSL)

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

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

Table 4.4. Volatile Compounds in Rice Bran Oil and Rice Bran Oil Structured Lipid (RBOSL)

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

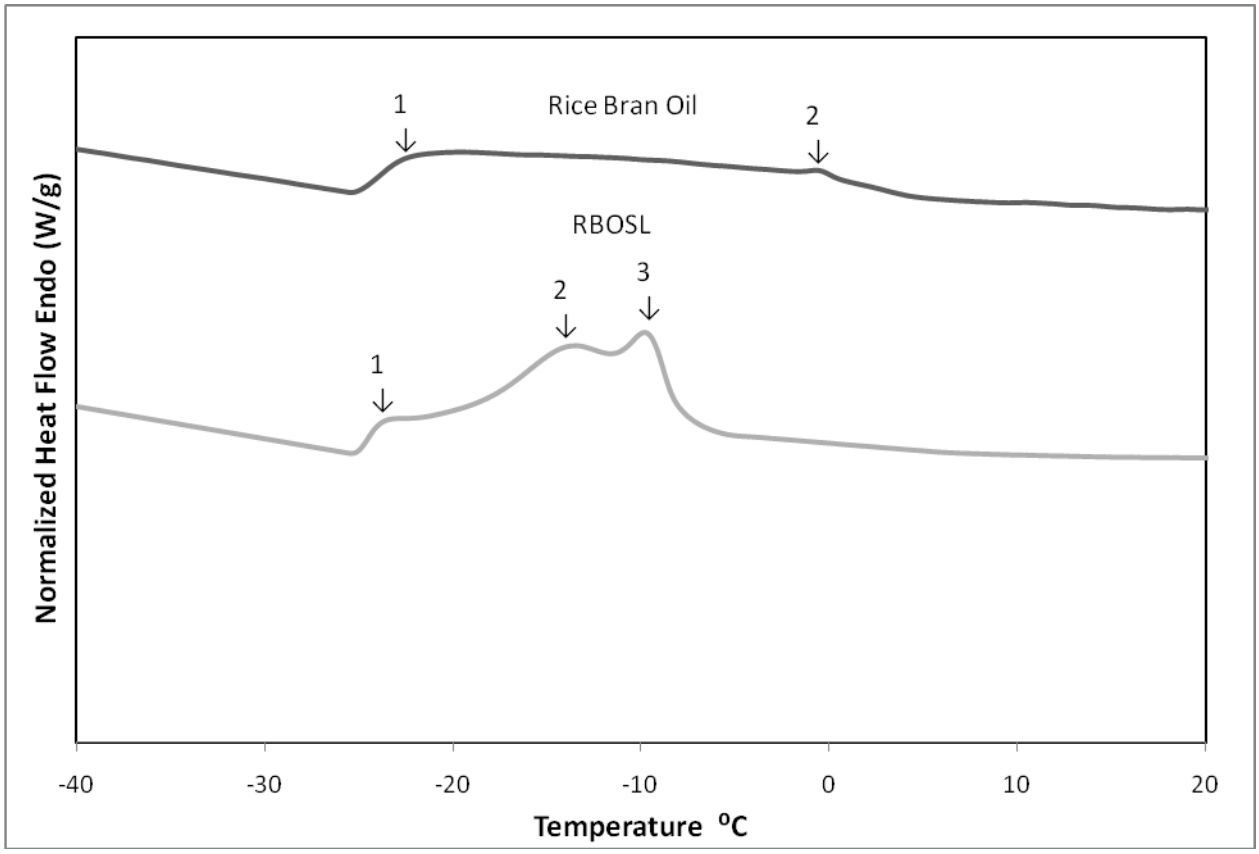
Table 4.5. Differential Scanning Calorimetry (DSC) Melting Properties of Rice Bran Oil and Rice Bran Oil Structured Lipid (RBOSL)

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±0.4B	81.7±0.1A

Mean ± SD, n=2; means with the same letter in the same row are no significantly different (P≤0.05).

Figure 4.1. Differential scanning calorimetry (DSC) thermograms of rice bran oil and rice bran oil structured lipid (RBOSL).

Figure 4.1.


Rice Bran Oil
RBOSL
1
2
1
2
3
Normalized Heat Flow Endo (W/g)
-40
-30
-20
-10
0
10
20
Temperature °C

CHAPTER 5

FOOD APPLICATIONS OF A RICE BRAN OIL STRUCTURED LIPID IN FRIED SWEET POTATO CHIPS AND AN ENERGY BAR

Jennings B.H., Shewfelt R.L. and Akoh, C.C. submitted to Journal of Food Quality

ABSTRACT

A structured lipid (RBOSL) was synthesized from rice bran oil (RBO) and caprylic acid with Lipozyme RM IM as biocatalyst. Sweet potato chips (SPC) were fried separately in RBOSL and RBO. Energy bars (EB) were formulated with RBOSL or RBO. Triangle tests (TT) was conducted for SPC and EB to determine panelists' ability to differentiate between SPC and EB prepared with RBO or RBOSL. Willingness to purchase (WTP) sensory analysis was also conducted. Fatty acid content, γ-oryzanol, viscosity, FFA and p-anisidine values for RBO and RBOSL were determined before and after frying. SPC color and oil uptake were also determined. TT results for SPC showed no significant difference in SPC fried in RBO and RBOSL ($P>0.05$). TT results for EB indicated a significant difference between RBO and RBOSL formulations ($P\leq0.05$). WTP (five point scale) sensory analysis of SPC and EB showed the most frequent response was probably would buy.

PRACTICAL APPLICATIONS

Consumer panels indicated a willingness to purchase both sweet potato chips and energy bars prepared with RBOSL. Improvements in standardization and preparation procedures and changes in formulation would probably improve acceptability. These consumer panel results showed that products prepared with SLs with improved nutritional or health promoting properties may have viable commercial potential in foods. RBOSL that is liquid at room temperature can potentially be used in frying and baking applications.

INTRODUCTION

Structured lipids (SLs) are produced by modifying triacylglycerols (TAGs) from their natural state to incorporate new fatty acids or to change the position of fatty acids or the fatty acid profile (Akoh and Kim 2008). Medium chain fatty acids (MCFAs) are metabolized through the portal system instead of the lymphatic system and provide quick energy sources that can be rapidly oxidized and utilized by the body. They are often incorporated into TAGs to produce SLs. MCFAs have been used by athletes requiring high energy diets and to treat patients with fat absorption abnormalities (Kennedy 1991; Megremis 1991). TAGs containing MCFA used in animal and human studies have been shown to increase energy expenditure and decrease weight gain (St-Onge and Jones 2002; St-Onge *et al*. 2003). The health promoting properties of MCFAs incorporated into SLs are related to their mobility, solubility and ease of metabolism. MCFAs are often targeted at the *sn*-1,3 positions of SLs because they are easily hydrolyzed and not stored as fat, whereas those at the *sn*-2 position are more easily absorbed and often contain essential long chain fatty acids (LCFAs) (Jandacek *et al.* 1987). The health benefits of SLs can include improved immune function, reduction of cholesterol, improved nitrogen balance and reduction in cancer risk (Akoh and Kim 2008).

Previous studies have examined the conditions for the production of a rice bran oil structured lipid (RBOSL) (Jennings and Akoh 2000), oxidative stability (Jennings and Akoh 2009a) and characteristics of (Jennings and Akoh 2009b) a rice bran oil structured lipid (RBOSL) enzymatically modified to contain capric or caprylic acid. There have been no studies that evaluated the sensory properties and consumer acceptance of a RBOSL used in food applications. RBOSL that is liquid at room temperature can potentially be used in butter and

margarine blends, frying and baking applications, and in beverages. Information on sensory properties of a RBOSL could possibly lead to increased interest in and use of SL in the food industry. Few studies on the sensory properties and consumer acceptability of SLs containing MCFAs have been done. Osborn *et al.* (2003) conducted sensory analysis of a nutritional beverage containing a canola oil/caprylic acid SL with a triangle test and qualitative descriptive analysis (QDA). Kim *et al.* (2005) conducted sensory analysis of a butterfat-vegetable oil blend spread prepared with SL containing canola oil and caprylic acid by conducting a triangle test and QDA to determine the effect of SL on the sensory profile of the spread.

RBO contains γ-oryzanol, which is a ferulic acid ester of sterols and terpene alcohols. γ-Oryzanol has been found to lower plasma cholesterol (Rukmini and Rughuram 1991), reduce cholesterol absorption (Seetharamaiah and Chadrasekhara 1990) and inhibit platelet aggregation (Seetharamaiah *et al*.. 1990). Jennings and Akoh (2009 a), determined that the γ-oryzanol concentration of RBOSL containing caprylic acid was not significantly different from unmodified RBO.

Frying remains a common means of food preparation that produces a very desirable color, flavor and texture. During deep fat frying food is cooked via heat transfer from the oil to the food and there is drying due to mass transfer, with both processes occurring simultaneously. Aeration, oxidation, absorption, vaporization, hydrolysis, and polymerization are among the physical and chemical changes that occur during frying. These chemical reactions and reaction products contribute to distinctive fried flavor, brown color and crisp texture of fried foods with the type of oil, frying conditions and degradation products affecting flavor quality (Warner 2008). The most desirable frying oils give good flavor to fried foods and have greater oxidative stability and fry life. The low linolenic acid content of rice bran oil allows good storage stability

and fry life (McCaskill and Zhang 1999). Valhalla *et al.* (2004) assessed rice bran oils as a cooking medium and found it to be generally well accepted. Information on the suitability of RBOSL as a frying medium compared to RBO would be very useful and would add significantly to this limited area of knowledge. Medium chain triacylglycerols (MCT) are not generally suitable for frying because of their low smoke point and high foaming tendencies; however, SLs enzymatically modified to contain MCFA and long chain fatty acids (LCFA) could decrease foaming and increase the smoke point (Negishi 2005). There have been few studies that evaluated SLs containing MCFA and LCFA as frying medium. Negishi *et al.* (2003) determined the relationship between the amount of foaming during frying and molecular weight of oils containing MCFAs. The study found that some canola oil containing MCFAs (either physical mixtures or esterified) had low foaming properties and were suitable for frying.

Sweet potato chips were chosen as one food application of RBOSL for this frying study because sweet potatoes are high in nutritional value. They provide 262.2% daily value (DV) of vitamin A (in the form of beta carotene), 12.6% DV of fiber, 28.4% DV of vitamin C, and 8.1% DV of iron (www.whfoods.org). Energy bars were chosen as a second food application of RBOSL because they were originally created as fuel for athletes and body builders and a SL containing MCFA would possibly be more beneficial than conventional fats usually contained in these products. Energy bars are also known as nutrition bars, sports nutrition bars, granola bars and meal replacement bars (Painter and Prisecaru 2002) and although convenient, are not believed to be a substitute for a healthy diet and proper nutrition. Energy bars offer some advantages over candy because they are lower in fat and sugars and higher in fiber and may also be fortified with vitamins and minerals. Bars that provide real food such as whole grains and dried fruits are recommended over others (Hurley and Leibman 2000). Foods formulated with

higher carbohydrate concentrations cause more extreme fluctuations in blood sugar and can lead to insulin resistance, diabetes and weight gain, whereas foods formulated with added protein and fat result in more gradual increases in blood sugar (Painter and Prisecaru 2002).

Enzymatically modified RBO containing caprylic acid (C8:0) at the *sn*-1,3 positions and with primarily oleic and linoleic acids at the *sn*-2 position were used in this study. The positional specific lipase, Lipozyme RM IM, was used to catalyze the enzymatic modification reaction (Jennings and Akoh 2009b). Our objectives were: 1) to substitute a SL containing rice bran oil and caprylic acid to provide a healthier alternative to other fats currently used in energy bars and as a fat source that can be burned quickly for energy, and 2) to conduct sensory evaluation of sweet potato chips fried in RBOSL and an energy bar formulated with RBOSL. Both foods were evaluated by a triangle test to determine if panelists could differentiate between sweet potato chips and energy bars prepared with RBO or RBOSL. A willingness to purchase five point scale was used to determine consumer acceptance of sweet potato chips and energy bars prepared with RBOSL in a separate test. In addition, the fatty acid profile of RBOSL was determined after frying sweet potato chips. Oil uptake, γ-oryzanol concentration, free fatty acid content, *p*-anisidine, color and viscosity were also determined for the sweet potato chip frying study.

MATERIALS AND METHODS

Materials

RBO 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 macroporous anion exchange resin) from *Rhizomucor miehei* was donated by

Novozymes North America, Inc. (Franklinton, NC). All ingredients for energy bars except butter flavor were purchased from a local grocery store. Butter flavor was donated by (ButterBuds, Racine, WI). Sweet potatoes for the frying study were donated by the Louisiana Agricultural Experiment Station (Chase, LA).

Synthesis of Rice Bran Oil Structured Lipid

The SL was produced in 1 kg quantities in a packed-bed reactor with substrate flow rate of 1ml/min, 1:6 substrate mole ratio (rice bran oil:caprylic acid), and a temperature of 45 °C (Kim & Akoh, 2006). A bioreactor with a jacketed, stainless steel column (47 mm x 500 mm) and a FMI Lab 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 (2002). 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 (Jennings and Akoh 2009a).

Short-path Distillation

The SL reaction products were passed through a short-path distillation apparatus 4 times to remove free fatty acids to a level below 0.1 %. A KDL-4 (UIC Inc., Joliet, IL) distillation unit was used to purify the reaction products by separating the FFA from the TAG. The heating oil temperature was 185 °C, the coolant temperature was 15 °C, and the vacuum pressure was below 1 Torr (Fomuso and Akoh 2002).

Energy Bar Formulation

The ingredients in the energy bar were as follows: whole wheat flour 241.2 g, sucralose 13.5g, sugar 57.0 g, brown sugar 30.6 g, skim milk powder 33.0 g, cinnamon 2.0 g, butter flavor, 14.5 g, egg 53.8 g, RBO or RBOSL, 197.2 g, applesauce 36.2 g, white grape juice, 245.5 g, vanilla extract, 8.3g, dried cranberries, 128.0 g, raisins, 124.0 g, sliced almonds 91.3 g, and oats 209.5 g. The bars were baked at 176 °C for 30-40 min. This recipe is based on a recipe by Painter and Prisecaru (2002) for complex carbohydrate bars, which included canola oil, whole wheat flour, sugar, brown sugar, vanilla extract and egg. Other ingredients and ingredient amounts added to the energy bars for this study were modifications.

Sweet Potato Chip Frying Study

A frying study was carried out to determine suitability of rice bran oil SL as a frying medium. Sweet potato chips were evaluated after frying at 165-185 °C separately in RBO and RBOSL. Sweet potatoes were soaked in an 8% boiling NaOH solution for 4 min and rinsed thoroughly with water and scraped to remove the skin. The sweet potatoes were then rinsed again and cut into slices of approximately 1.0-2.0 mm thickness. The chips were washed again to remove free starch, blotted with a paper towel, and dried at 60 °C for 25-30 min. The sweet potato chips were fried for 20-60 s at 165-185 °C (Singh *et al.*, 2003). A Dazey model DTC-1 (Airport, KS) fryer was used with an oil volume of 2 L. The total time to complete frying of all chips for the day's sensory analysis did not exceed 30 min and was completed with no more than 10 batches of 30 - 40 chips. RBO and RBOSL were not reused after all batches were completed for the day. The chips were then placed on a paper towel to drain the oil and placed

in an oven at 110 °C for 10 min. Salt (6mg/g chips) was then sprinkled onto the chips, which were then shaken to distribute the salt.

Free fatty acid content, p-anisidine value, viscosity and oil uptake was measured and results were compared between modified and unmodified rice bran oil. FFA and p-anisidine values were determined according to AOCS Official Methods (1997) Ca-5a-40 and Cd-18-90 respectively. Color of sweet potato chips was measured with a Minolta CR-300 Chroma Meter (Osaka, Japan) with la Commission Internationale de l'Eclairage (CIE) L*a*b* (for lightness, redness and yellowness, respectively) color system. Sweet potato chip oil uptake was determined by Soxhlet extraction following AOAC official method 920.39. Viscosity at 22.8 °C was determined with a RV Brookfield Digital Viscometer (spindle #2) (Brookfield Engineering Laboratories, Inc., Stoughton, MA).

Fatty Acid Profile

Gas liquid chromatography (GLC) was used for the fatty acid profile analysis of RBOSL before and after frying to determine the effect of frying on caprylic acid and other fatty acid concentrations. Fatty acid methyl esters were prepared as described by Jennings and Akoh (2000). The gas chromatograph was an Agilent 6890N (Wilmington, PA) equipped with an AT-225 fused-silica capillary column 30 m x 0.25 mm i.d. (Alltech, Deerfield, IL), flame-ionization detector, and operated in a 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 programmed to 215 °C for 10 min at a rate of 20 °C /min. The carrier gas was helium and the total gas flow rate was 23 ml/min. The relative concentration of FAME as mol% was calculated by computer with 17:0 as internal standard.

γ-Oryzanol Analysis

HPLC analysis to determine γ-oryzanol content of rice bran oil before and after modification and frying was performed with an Agilent (Wilmington, PA) model 1100 liquid chromatograph equipped with a diode array UV-visible detector. γ-Oryzanol standards were prepared from γ-oryzanol powder purchased from TCI America (Portland, OR). The stationary phase was a Phenomenex (Torrence, CA), ODS-2, RP C_{18} column. The initial mobile phase conditions were 45% acetonitrile, 40% methanol, 5% isopropyl alcohol, and 10% aqueous acetic acid. After 4 min, the mobile phase was changed linearly to 25% acetonitrile, 70% methanol, 5% isopropyl alcohol for 10 min and held for 11 min with a total run time of 25 min. After each run, the solvent gradient was changed linearly to 95% methanol and 5% isopropyl alcohol and held for 1 min before returning to initial mobile phase conditions. γ-Oryzanol peaks were detected at 325 nm and quantified from a standard curve (Chen and Bergman 2005).

Sensory Analysis

The panel for the triangle test consisted of 30-36 students and staff from the University of Georgia Campus. All panelists consented to participate by signing a form approved by the University of Georgia Institutional Review Board.

The triangle test (Meilgaard *et al.* 2007) was used to determine if the panelists given 3 samples could detect the sample that was different from the other 2 samples. Samples were either fried (sweet potato chips) or formulated (energy bar) with RBO or RBOSL. Sensory analysis of sweet potato chips and energy bars were conducted separately.

Panel acceptability of samples containing RBOSL was also evaluated in sensory testing conducted on a separate day from triangle testing. Separate consumer tests were conducted to evaluate the RBOSL used in an energy bar and sweet potato chips fried in RBOSL. A total of 100-102 students and staff from the University of Georgia Campus rated the samples on a 5 point willingness to purchase scale (Moskowitz *et al.* 2005).

Statistical Analysis

Statistical analysis was conducted with the SAS software package (SAS Inst. 2000). Triangle test data was analyzed by matching the number of correct responses from the number of trials conducted to a probability table (Meilgaard *et al,* 2007). A t-test was used to determine the differences between 2 samples (RBOSL and unmodified RBO as well as before and after frying). Statistical differences with $P \leq 0.05$ were considered significant.

RESULTS AND DISCUSSION

Chemical and Physical Characteristics

Table 5.1 shows the fatty acid profile of RBOSL before and after frying sweet potato chips. Mol% fatty acids for caprylic, palmitic, stearic, oleic and linoleic acids were not significantly different after frying ($P>0.05$). These findings clearly show that the volatile nature of caprylic acid is not a concern in a triacylglycerol when subjected to frying temperatures. Table 5.2 shows some chemical characteristics of RBOSL before and after frying. Free fatty acid content and p-anisidine value are indicators of oxidative stability. Free fatty acids are formed due to the reaction of air and water, which causes hydrolysis of fatty acids from TAG during frying. The p-anisidine value measures the amount of aldehydes present in oil.

Aldehydes such as 2-alkenals and 2,4-alkadienals are secondary oxidation products formed from the decomposition of peroxides. The free fatty acid content of 0.22±0.01 and 0.33±0.01 % and *p*-anisidine values of 16.52±1.15 and 28.29±1.76 mg/kg were significantly higher ($P \leq 0.05$) in RBOSL than in RBO after frying sweet potato chips (Table 5.3). Jennings and Akoh, 2009a found that the OSI values of 12.40±0.20 for RBO and 11.40±0.00 for RBOSL were significantly different. Our results show that a heat stable antioxidant may need to be added to RBOSL before use in a frying application.

The concentrations of γ-oryzanol before frying were 6999.94±64.09 µg/ ml for RBO and 7377.26±7.61 µg/ml for RBOSL and were not significantly different ($P>0.05$) (Table 5.2). γ-Oryzanol concentration of RBO (6493.18 ±120.26 µg/ml) and RBOSL (6296.07±2.46 µg/ml) was also not significantly different after frying ($P>0.05$) (Table 5.3). The γ-oryzanol concentrations for RBO and RBOSL before frying were slightly higher than after frying. Khuwijitjaru *et al.* (2004) described the degradation kinetics of γ-oryzanol by the first order reaction model at 120, 150 and 200 °C and reported degradation rate constants of 0.0089, 0.0315 and 0.0763, respectively. Krishna *et al.* (2005) however, found that the γ-oryzanol content of RBO was not affected after frying up to 1 hr at 180 °C. The health benefits and oxidative stability of RBO is due in large part to the γ-oryzanol content. These results demonstrate that frying or enzymatic modification has little or no effect on the γ-oryzanol concentration of RBOSL.

Viscosity values at 22.8 °C before frying of 66.80±0.15 and 78.6±0.20 for RBOSL and RBO respectively were significantly different ($P \leq 0.05$) (Table 5.2). The viscosity of RBOSL (60.90±0.00 cP) was significantly lower ($P \leq 0.05$) than RBO (81.60±0.00 cP) after frying sweet potato chips (Table 5.3). Oil uptake in RBOSL (19.48±0.15 %) was also significantly lower

than RBO (26.16±0.16 %) ($P \leq 0.05$) (Table 5.3). The lower viscosity of RBOSL which is due to the incorporation of lower viscosity caprylic acid causes decreased surface contact and higher interfacial tension and therefore resulted in lower oil uptake (Gillatt 2001). During frying of the sweet potato chips, more foaming was observed in the RBOSL than in RBO. However, the foam remained contained in the fryer during the frying and did not interfere with the frying process. Similar findings were reported by Negishi (2003), showing that canola oil containing MCFAs in a physical mixture or esterified had low foaming properties and were suitable for frying. Jennings and Akoh (2009b) reported that the smoke point of RBOSL (253.00±3.00 °C) was not significantly different from RBO (257.00±4.00 °C). Table 5.4 shows the color variables for sweet potato chips fried in RBO compared to those fried in RBOSL. None of the color variables measured was significantly different for RBO and RBOSL.

Sweet Potato Chip and Energy Bar Triangle Tests

A triangle test was conducted to determine if panelists could determine differences in sweet potato chip samples fried in RBO versus RBOSL. Triangle test results for sweet potato chips fried in RBO compared to sweet potato chips fried in RBOSL showed that 14 out of 30 panelists correctly identified the chip fried in RBOSL. The results revealed that RBO and RBOSL fried sweet potato chips were not significantly different ($P > 0.05$).

Triangle test results for the energy bar formulated with RBO compared to the energy bar formulated with RBOSL revealed that 18 out of 36 panelists correctly identified the RBOSL energy bar formulation and were significantly different ($P \leq 0.05$). Panelists who correctly identified the odd sample also reported that the RBO formulation had a softer texture than the RBOSL formulation.

A triangle test conducted on a nutritional beverage containing a canola oil/caprylic acid SL by Osborn *et al.* (2003) reported that 23 out of 38 panelists were able to identify the odd sample containing canola oil/SL versus the sample containing canola oil alone, which indicated a significant difference between samples. QDA of the nutritional beverage containing a canola oil/caprylic acid SL revealed that substituting the canola oil/caprylic acid SL for unmodified canola oil enhanced the perception of sweet flavor and decreased bubble formation; other attributes were unchanged. Sensory analysis of a butterfat-vegetable oil blend spread prepared with a SL containing canola oil and caprylic acid was conducted by Kim *et al.* (2005). Sensory analysis included triangle test and QDA to determine the effect of SL on the sensory profile of the spread. Triangle test results of pure butter versus butterfat-SL blend indicated that there was a significant difference between the samples ($P \leq 0.05$). QDA indicated that the blend containing SL was significantly more cold spreadable than pure butter with a textural profile similar to the sample without SL. Significant differences were not found between the spread samples flavor attributes. These results suggest that other foods can possibly be formulated with SL containing caprylic acid without adversely affecting flavor and texture. Our triangle test results for the energy bar formulated with RBO or RBOSL showed that there was a significant difference between formulations ($P \leq 0.05$) which were in agreement with triangle test results on the sensory evaluation of food products containing a SL by Osborn *et al.* (2003) and Kim *et al.* (2005).

Willingness to Purchase Panels

More than 50% of the panelists were willing to purchase sweet potato chips (definitely or probably would buy) (Figure 5.1). Some panelists reported that salt distribution was uneven with

comments ranging from too salty to not salty enough. Panelists also indicated that some chips were soggy or chewy *et al*. indicated that the chips were crispy. Differences in consistency may have been related to variations in chip thickness and frying time. The chip slice thickness and frying time should be optimized and textural analysis should be used for sweet potato chips fried in RBOSL in future studies to give a more uniform crispy texture. The method of seasoning distribution should also be improved. More than 60% of the 102 total panelists reported that they were willing to purchase the energy bar formulated with RBOSL (Figure 5. 2). Most panelists had an overall favorable impression of the product and indicated that they liked the texture, aroma and taste. Other panelists' comments were that the texture was dry and grainy. The energy bar samples from the edges of the baking pan had a drier texture than those from the interior of the pan. The energy bar formulation and baking process should be improved in future studies for a more uniform moist texture.

CONCLUSIONS

The fatty acid profile of RBO compared to RBOSL after frying was almost unchanged. The color of sweet potato chips after frying in RBOSL was not significantly different from those fried in RBO. γ-Oryzanol concentration of RBOSL after frying was not significantly different from RBO. Free fatty acid content and *p*-anisidine values were significantly higher in RBOSL than RBO after frying. Oil uptake was significantly lower in RBOSL than in RBO. Results of triangle tests indicated that there was not a significant difference in sweet potato chips and a significant difference in energy bars prepared with RBOSL compared to RBO. Consumer panels indicated a willingness to purchase both sweet potato chips and energy bars but greater standardization and changes in formulation would probably improve acceptability. These

consumer panel results were very encouraging and indicate that products prepared with SLs with improved nutritional or health promoting properties may have viable commercial potential.

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Table 5.1. Fatty acid profile (mol%) of rice bran oil structured lipid (RBOSL) before and after frying sweet potato chips at 185 °C.

Fatty acid	Before frying	After frying
8:0	33.08±1.39A	32.58±0.61A
16:0	10.89±0.20A	11.11±0.03A
18:0	0.98±0.18A	1.03±0.02A
18:1	29.59±1.48A	26.02±5.91A
18:2	25.58±0.30A	25.94±0.43A

Mean ± SD, n=3 means with the same letter in the same row are not significantly different ($P > 0.05$)

Table 5.2. Chemical characteristics of rice bran oil (RBO) and rice bran oil structured lipid (RBOSL) before frying sweet potato chips

Characteristic	Before frying in RBO	Before frying in RBOSL
Chip oil uptake (%)	-	-
γ-oryzanol (µg/ml)	6999.94±64.09A	7377.26±7.61A
free fatty acids (% as oleic)	0.10±0.00A	0.10±0.00A
p-anisidine (mg/kg)	11.20±0.04B	17.60±0.03A
viscosity (cP) (22.8 °C)	78.60±0.20A	66.80±0.15B

Mean ± SD, n=2 means with the same letter in the same row are not significantly different (P > 0.05)

Table 5.3. Chemical characteristics of rice bran oil (RBO) and rice bran oil structured lipid (RBOSL) after frying sweet potato chips

Characteristic	After frying in RBO	After frying in RBOSL
Chip oil uptake (%)	26.16±0.16A	19.48±0.15B
γ-oryzanol (µg/ml)	6493.18±120.26A	6296.07±2.46A
free fatty acids (% as oleic)	0.22±0.01B	0.33±0.01A
p-anisidine (mg/kg)	16.52±1.15B	28.29±1.76A
viscosity (cP) (22.8 °C)	81.60±0.00A	60.90±0.00B

Mean ± SD, n=3 means with the same letter in the same row are not significantly different (P > 0.05)

Table 5.4. Color values of sweet potato chips (SPC) after frying in rice bran oil (RBO) and rice bran oil structured lipid (RBOSL)

Values	SPC after frying in RBO	SPC after frying in RBOSL
L*	36.71±0.01A	36.33±1.00A
a*	10.41±0.49A	11.85±1.60A
b*	28.88±0.27A	29.95±0.32A
C	30.68±0.33A	31.05±1.47A
h°	70.18±1.52A	67.49±2.98A

Mean n=3; means with the same letter in the same row are not significantly different ($P \leq 0.05$).

FIG. 5.1. PLOT OF WILLINGNESS TO PURCHASE CONSUMER ACCEPTANCE PANEL FOR SWEET POTATO CHIPS FRIED IN RICE BRAN OIL STRUCTURED LIPID

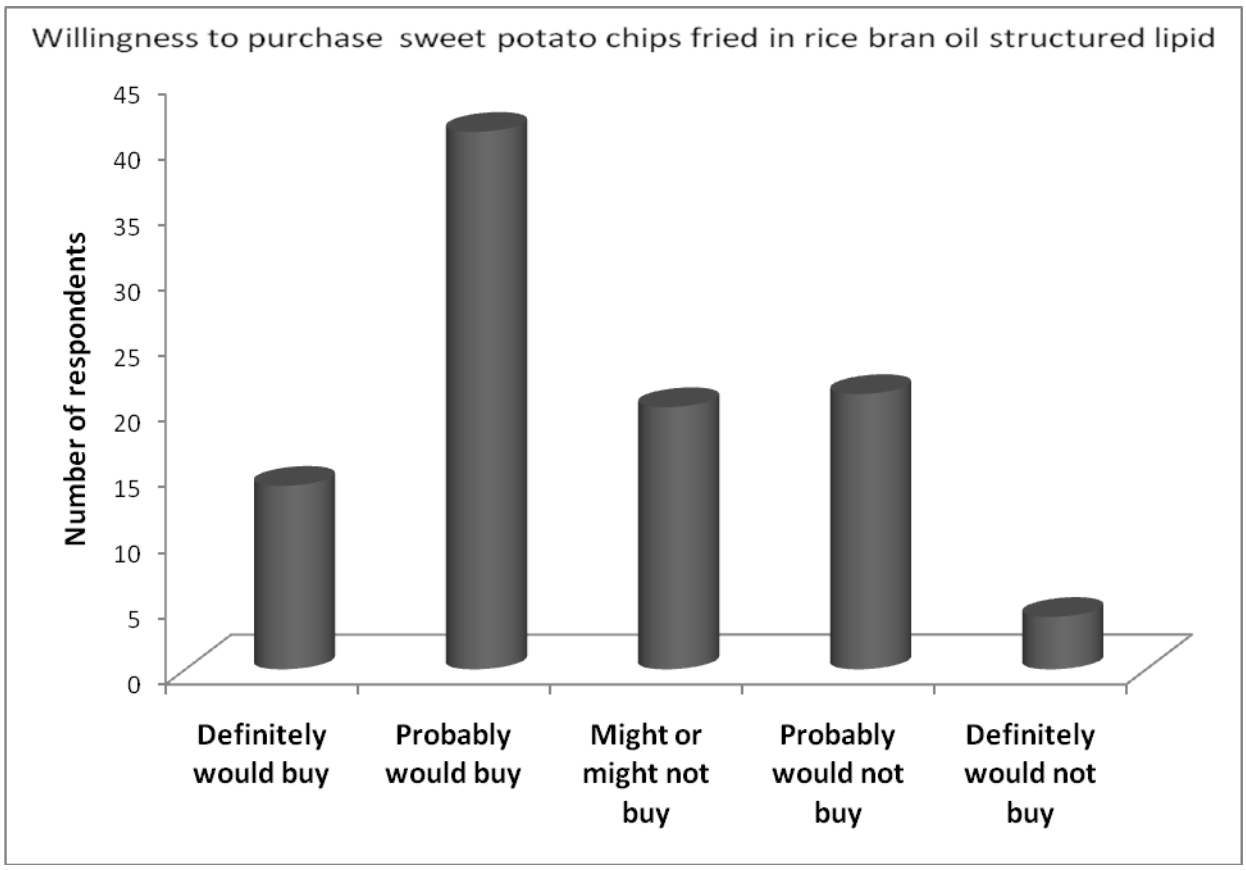
Willingness to purchase sweet potato chips fried in rice bran oil structured lipid
Number of respondents
45
40
35
30
25
20
15
10
5
0
Definitely would buy
Probably would buy
Might or might not buy
Probably would not buy
Definitely would not buy
FIG. 5.1.

FIG. 5.2. PLOT OF WILLINGNESS TO PURCHASE CONSUMER ACCEPTANCE PANEL FOR ENERGY BAR FORMULATED WITH RICE BRAN OIL STRUCTURED LIPID

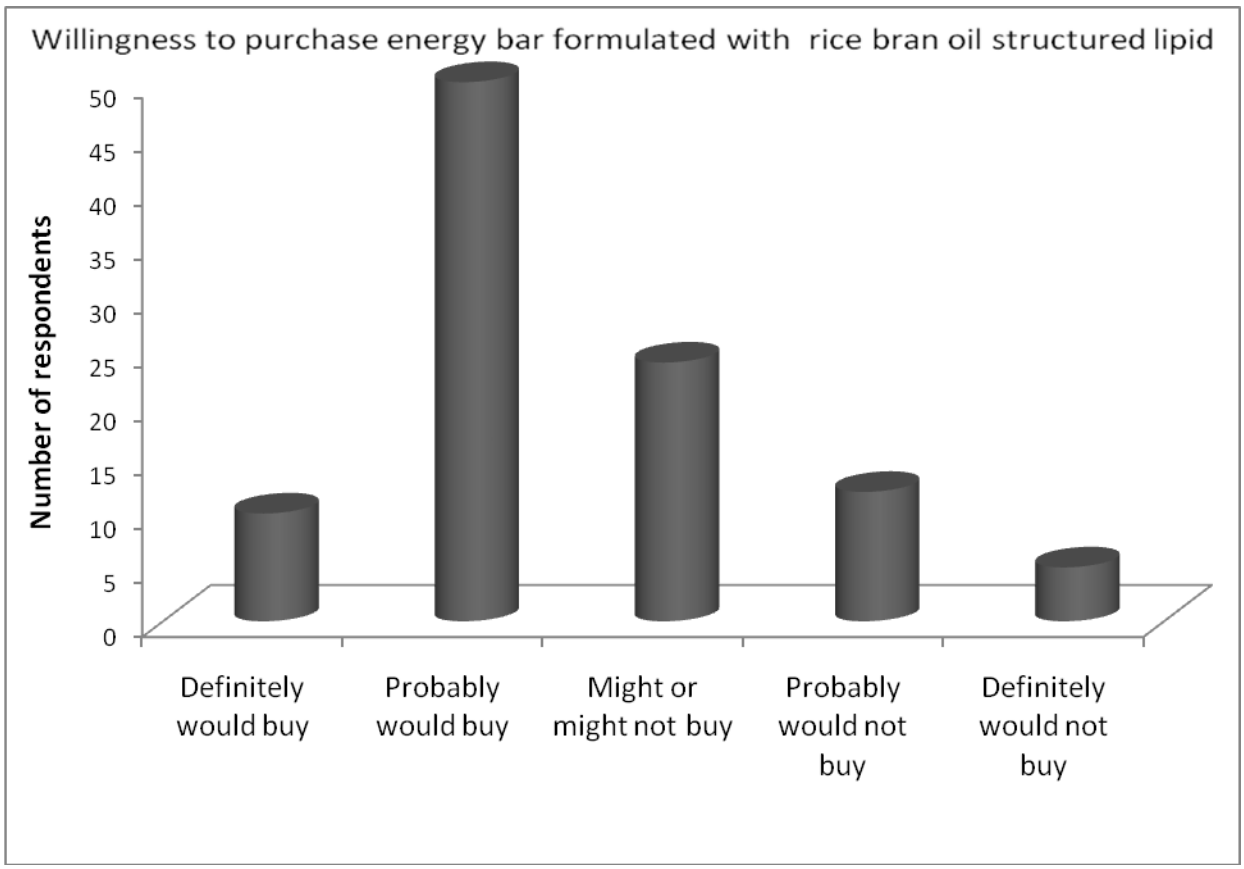
Willingness to purchase energy bar formulated with rice bran oil structured lipid
Number of respondents
50
45
40
35
30
25
20
15
10
5
0
Definitely would buy
Probably would buy
Might or might not buy
Probably would not buy
Definitely would not buy

FIG.5. 2.

CHAPER 6

TRANS-FREE PLASTIC SHORTENINGS PREPARED WITH PALM STEARIN AND RICE BRAN OIL STRUCTURED LIPID

Jennings BH, Akoh CC submitted to the Journal of the American Oil Chemists' Society

Abstract Rice bran oil structured lipid (RBOSL) was produced from rice bran oil (RBO) and the medium chain fatty acid (MCFA), caprylic acid, with Lipozyme RM IM as biocatalyst. RBOSL and RBO were mixed with palm stearin (PS) in ratios of 30:70, 40:60, 50:50, 60:40 and 70:30, v/v (RBOSL to PS) to formulate *trans*-free shortenings. Fatty acid profiles, solid fat contents (SFC), melting and crystallization curves, and crystal morphology were determined. The content of caprylic acid in RBOSL ranged from 9.92 to 22.14 mol%. Shortening blends containing 30:70 and 60:40 RBOSL or RBO and PS had fatty acid profiles similar to a commercial shortening (CS). SFCs for most blends were within the desired range for CS of 10-50% at 10-40 °C. Shortening blends containing higher amounts of RBOSL or RBO had melting and crystallization curves similar to CS. All shortening blends contained primarily β' crystals. RBOSL blended with PS was comparable to RBO in producing shortenings with fatty acid profiles, SFC, melting and crystallization profiles, and crystal morphologies that were similar. RBOSL blended with PS can possibly provide healthier alternative to some oils currently blended with PS and commercial shortening to produce *trans*-free shortening because of the health benefits of the MCFA in RBOSL.

Keywords Palm stearin · Rice bran oil · Shortening · Solid fat content · Structured lipid

Introduction

Shortening provides desirable textural properties by lubricating, weakening, or shortening food components. Shortening used in frying allows uniform heat transfer and forms a moisture barrier. Other desirable functions of shortening in food include, imparting tenderness and mouth feel, providing structural integrity, incorporation of air, and extending shelf life [1].

Shortening is produced from hydrogenated vegetable oils. The hydrogenation process produces *trans* fatty acids, which when consumed can increase the risk for coronary heart disease [2]. Fractionation and blending of palm oil with RBO and mahua oil [3], with palm stearin (PS) and RBO [4] has resulted in *trans*-free shortenings. Interesterification of RBO and PS has also produced *trans*-free shortenings [5, 6]. Another alternative to blending palm oil fractions with vegetable oil is to blend palm oil fractions with a structured lipid (SL).

Palm oil is high in palmitic, oleic, and linoleic acids and has a higher polyunsaturated fatty acid (PUFA) content than coconut and palm kernel oils. Palm oil is extracted from the mesocarp of the oil palm (*Elaeis guineensis*) fruit, is economical to produce and can be fractionated into liquid fraction (palm olein) and solid fraction (PS). The high oxidative stability of palm oil is due to its high tocopherol content. The carotenoids and tocopherols present in palm oil protect against certain cancers and lower serum cholesterol, respectively [7]. Palm oil does not promote atherosclerosis and arterial thrombosis although it contains 50% saturated fatty acids [7]. The solid fat content (SFC) of palm oil gives consistency without hydrogenation and can be used in food products with variable plastic ranges [7].

A SL containing RBO and the medium chain fatty acid (MCFA), caprylic acid, would have several health benefits and would contribute to the desirability of a shortening made by blending palm stearin with RBOSL. RBO contains γ-oryzanol which can lower plasma

cholesterol [8], cholesterol absorption [9], and inhibit platelet aggregation [10]. The γ-oryzanol concentration in RBO is not significantly affected by enzymatic modification to incorporate caprylic acid with Lipozyme RM IM as biocatalyst [11]. MCFAs provide a quick energy source that can be rapidly oxidized and utilized. MCFAs are metabolized through the portal system instead of the lymphatic system for long chain fatty acids. MCFAs have been used to treat patients with fat absorption abnormalities and by athletes with increased energy requirements [12, 13]. Previous animal and human studies have shown that the composition of triacylglycerols (TAGs) containing MCFA resulted in increased energy expenditure and decreased weight gain [14, 15]. Blending of RBOSL with PS is an option for producing *trans*-free shortening without hydrogenation and with the added health benefit of MCFA contained in RBOSL.

The objectives of this study were: 1) to blend RBOSL or RBO with PS in various ratios to produce shortenings, and 2) to determine the fatty acid profile, melting and crystallization profile, SFC, and crystal morphology of the blended shortening and commercial shortenings (CS).

Experimental Procedures

Materials

RBO 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 macoporous anion exchange resin) from *Rhizomucor miehei* was purchased from Novo Nordisk Biochem North America, Inc. (Franklinton, NC). RBD PS was donated by Fuji Vegetable Oil, Inc. (Savannah, GA). CS #1 (palm oil shortening) was purchased from Loders

Croklaan North America, LLC (Channahon, IL). CS #2 (hydrogenated vegetable oil shortening) was purchased from a local grocery store.

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 (RBO: caprylic acid) and a temperature of 45 °C [11, 16]. A bioreactor with a jacketed stainless steel column (47 mm x 500 mm) and a FMI Lab 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 [17]. 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 4 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 [17].

Determination of Shortening Fatty Acid Profiles

One hundred milligrams of the shortening sample was weighed into a culture tube and 1 mL of internal standard (17:0 in hexane 20 mg/mL) was added to the culture tube and the mixture was then flushed with nitrogen. Two milliliters of 0.5N NaOH in methanol solution was then added and the samples were placed in an oven at 100 °C for 5 min. Two milliliters of 14% BF_3 in methanol was added and the reaction mixture was vortexed for 1 min and again placed in an

oven at 100 °C for 5 min. Two milliliters of hexane and 2 mL of saturated NaCl was then added to extract the fatty acid methyl esters (FAMEs). The samples were then vortexed for 2 min and centrifuged at 1000 rpm for 5 min at room temperature [18]. The upper layer was then removed and analyzed by gas chromatography. The gas chromatograph was an Agilent 6890N (Wilmington, PA) equipped with a Supelco (Bellefonte, PA) SP-2560 100 m x 0.25 mm x 0.20 µm film thickness column and a flame ionization detector operated in the split mode with a split ratio of 50:1. The injector and detector temperatures were both at 250 °C. The column temperature was at 140 °C. The carrier gas was helium and the flow rate was 1.1 mL/min. The relative concentrations of FAMEs were calculated by computer with 17:0 as internal standard. Retention times of GLC reference standard (Supelco 37, Bellefonte, PA) were used to identify detected FAMEs.

Shortening Blend Preparation

PS was melted at 65 °C and was blended with RBOSL in ratios of (RBOSL or RBO to PS) 30:70, 40:60, 50:50, 60:40 and 70:30, v/v, to produce shortenings.

Differential Scanning Calorimetry

A Perkin-Elmer model DSC7 (Norwalk, CT) was used for the analysis of melting profiles according to AOCS recommended procedure Cj 1-94 [19]. Indium was used as a reference standard and for standardization (mp 156.6 °C, ΔH 28.45 J/g); dry ice was used as a coolant. The 5-20 mg sample was hermetically sealed in 30 µL capacity aluminum pan (Perkin Elmer); an empty pan was used for reference. Samples were heated rapidly from room temperature to 80 °C and held for 10 min (to destroy crystal memory). The sample was then cooled to - 40 at 10 °C per min, held for 30 min, and then heated to 80 °C at a rate of 5 °C per min to generate

melting profiles. The thermograms were analyzed by the software provided with the DSC (Pyris software, Perkin-Elmer, Shelton, CT).

Solid Fat Content

SFC was determined for shortening containing unmodified RBO blended with PS, and shortenings containing RBOSL blended with PS and CS by AOCS Official method Cd 16-81 [19]. NMR measurements were made using a MARAN-20 pulsed NMR spectrometer (Resonance Instruments Ltd., Oxon, United Kingdom). Samples were tempered at 100 °C for 15 min and placed at 60 °C for 10 min followed by 0 °C for 60 min and finally 30 min at each chosen measuring temperature. Olive oil was used as the reference oil. The SFC was measured at 5 °C intervals from 5 to 60 °C.

X-Ray Diffraction Spectroscopy

The polymorphic forms of shortening containing RBOSL blended with PS, shortening containing unmodified RBO blended with PS, and CS were determined to characterize and differentiate the crystal structures among the shortenings. Samples were kept at 24 °C and spread over a glass X-Ray slide. A Rigaku Multiflex (Rigaku Corporation, Tokyo, Japan) automated theta-theta powder X-Ray diffractometer with a copper X-Ray tube was used for the analysis. The generation power was 40 kV and 44 mA. The X-Ray analysis was performed at room temperature from 0.9° to 25° at a scan speed of 1°/min and a step of 0.02. MIDI Jade 6.5 software was used for data analysis.

Results and Discussion

Fatty Acid Composition

Table 6.1 shows the fatty acid profiles of shortening blends, CS, PS, RBO and RBOSL. Only CS #2 contained *trans* fatty acids (11.42 mol%). The major fatty acids for the shortening blends, commercial shortening and palm stearin were palmitic acid (14.07-58.75 mol%), oleic (25.52-38.20 mol%) and linoleic acids (7.10-30.03 mol%). Caprylic acid contents in blends containing RBOSL and PS ranged from 9.92 to 22.14 mol%. CS#2 and PS contained 0.42 and 1.55 mol% caprylic acid, respectively.

The saturated fatty acid content for blends containing RBO and PS ranged from 35.31-53.44 mol% and blends containing RBOSL and PS had saturated fatty acid contents of 48.38-59.70 mol%. Commercial shortenings had saturated fatty acid contents of 51.44 and 27.61 mol% for CS#1 and #2, respectively, and PS saturated fatty acid content was 67.01 mol%. RBO and RBOSL saturated fatty acid contents were 22.80 and 41.00 mol%, respectively. Overall, palmitic acid content decreased in PS after blending with RBO and RBOSL. Myristic, palmitic and stearic acid contents for blends containing 30:70 RBO or RBOSL were similar to those of CS#1. A previous study [4] reported that a 50:50 blend of RBO:PS had a saturated and unsaturated fatty acid contents of 41.00 and 53.20%, respectively. Our findings showed 44.06 mol% saturated fat and 55.94 mol% unsaturated fat for the 50:50 RBO:PS blend. The 50:50 RBOSL:PS blend contained 53.46 mol% saturated fat and 46.71 mol% unsaturated fat. The 70:30 RBOSL:PS blend had very similar and almost balanced saturated and unsaturated fatty acid contents of 48.38 and 51.68 mol%, respectively.

PS is highly saturated and provides natural hardness to fats, but this texture does not provide a suitable plastic range to edible fats such as margarine and shortening. Vegetable oils

such as RBO contain polyunsaturated fatty acids that are liquid at room temperature and when blended with PS helps to improve the fat plastic range.

Solid Fat Content

Determination of SFC allows a description of the amounts of solid fat crystals in relation to the amount of liquid oil from about 10-40 °C, which gives a measure of plasticity [20] and influences fat physical and sensory characteristics [21]. The plastic range of fat is the temperature range over which the fat can be molded and is neither too hard nor too soft. The 70:30 shortening blends containing 70:30 RBO or RBOSL and PS exhibited solid fat contents most similar to CS #1, which was a palm oil shortening (Fig. 6.1). The sharp decline in solid fat content for CS #1 shows a more limited plastic range than all other shortening blends; CS # 2 exhibited a less steep decline in solid fat content. The solid fat contents shown at 40 °C for the shortening blends were very similar to those reported in a previous study [21] with blends of sunflower oil and PS in nearly the same ratios (Fig. 6.1). A wider plastic range occurs when there is a more diverse fatty acid content with variable melting points. High stability shortening with a SFC of 10-50% at 10-40 °C is most desired [22]. The SFC for most shortening blends used in this study fell within this range, which indicates suitability for use in shortening applications requiring various consistencies. As the amount of PS increased in the shortening blends, the SFC also increased because of an increase in the saturated fatty acid content mainly from the palmitic acid content of palm stearin. The rate at which a fat solidifies can affect some bakery products such as pastries that must contain fats that become solid as the product cools to prevent fat loss which results in an oily paste [23].

Melting and Crystallization Properties

The melting profiles show that peak 3 for blends containing RBO and PS blends are very similar and only slightly higher than peak 3 for PS blends containing RBOSL (Fig. 6.2). As the content of RBO or RBOSL liquid oil increased, the height of peak 3 decreased and melting profiles for the blends became more similar to the CSs (Fig. 6.2). This change was due to the decrease in high melting TAG concentration and an increase in low melting TAG concentration. Similar findings were reported in a previous study of shortening blends containing PS and RBO [3, 4]. Peak 1a in the melting curve of the blends containing RBOSL is caprylic acid, which is distinctly absent in the melting peaks containing RBO and different from CS and PS (Fig. 6.2). The depressed area between peaks 1 and 2 (Fig. 6.2) is a recrystallization peak, which probably occurred when the α polymorph transitioned to the β' form [24]. This recyrstallization peak decreased as the amount of PS decreased.

Blends with RBOSL crystallized at slightly lower temperatures than blends containing RBO (Fig. 6.3). The lower crystallization temperature was because of the lower viscosity of RBOSL, which did not inhibit nucleation as much as the higher viscosity RBO [25]. As the concentration of palm stearin decreased, the size of the crystallization peak also decreased and the crystallization profiles became more similar to CS (Fig. 6.3).

Polymorphism

The three major polymorphic forms of fats consist of α, β and β' also referred to as hexagonal, orthorhombic, and triclinic, respectively. Shortenings are composed of a solid phase consisting of solid fat crystals and a liquid phase consisting of oil held together by cohesive forces [20]. Fat crystal size, shape, number and bonding force determine fat characteristics such as hardness and

plastic behavior [26]. The β' crystals, which have a smaller thinner structure, can hold more air and liquid component (an important functional quality of shortening) than larger, more stable, higher melting point β crystals. By comparison, the α crystal form has the lowest melting point and is the least stable of the polymorphs. The β' crystals provide a smother mouthfeel and are more desirable than the β crystal forms providing increased firmness and air incorporation [27].

X-Ray diffraction spectroscopy analysis (XRD) produced d spacings at 4.20 and 3.80 Å and at 4.15 Å. The peaks at 4.20 and 3.80 Å were much larger than those at 4.15 Å. The data showed that all shortenings and shortening blends tested in this study contained primarily β' crystals with β' > > α. These results demonstrate that RBOSL: PS blends form β' crystals as does the RBO: PS blend. The β' crystal form is more stable in shortenings with higher palmitic acid contents [28]. The PS used for this study contained 58.75 mol% palmitic acid and 30:70 and 40:60 blends containing RBO or RBOSL contained 39.58 - 47.49 mol% palmitic acid.

RBOSL blended with PS showed properties comparable to RBO:PS blends when the fatty acid profiles, solid fat contents, melting and crystallization profiles, and crystal forms were compared. Blending of RBOSL with PS is an option for producing *trans*-free shortening without hydrogenation and with the added health benefit of MCFA contained in RBOSL.

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Table 6.1. Fatty acid profiles of shortening blends, commercial shortenings, palm stearin, RBO and RBOSL

Shortening blend	Fatty acid								
	8:0	14:0	16:0	18:0	18:1c	18:1t	18:2c	18:3	20:0
30:70 RBOSL:PS	9.92±0.02	0.96±0.01	44.27±0.01	4.02±0.05	26.49±0.01	ND	13.58±0.04	0.36±0.02	0.53±0.04
30:70 RBO:PS	ND	1.06±0.03	47.49±0.01	4.37±0.01	30.39±0.01	ND	15.72±0.06	0.52±0.13	0.52±0.13
40:60 RBOSL:PS	12.44±0.06	0.89±0.06	39.58±0.01	3.65±0.01	28.30±1.90	ND	15.71±0.21	0.54±0.01	0.42±0.03
40:60 RBO:PS	ND	0.99±0.03	44.10±0.03	4.11±0.01	31.86±0.06	ND	17.87±0.01	0.60±0.14	0.69±0.04
50:50 RBOSL:PS	15.92±0.03	0.75±0.05	33.04±0.11	3.14±0.01	27.80±0.21	ND	18.39±0.01	0.52±0.06	0.61±0.06
50:50 RBO:PS	ND	0.88±0.04	38.78±0.01	3.90±0.03	33.74±0.01	ND	21.61±0.03	0.64±0.04	0.76±0.04
60:40 RBOSL:PS	19.12±0.01	0.64±0.06	28.06±0.03	2.75±0.06	27.85±0.01	ND	20.55±0.01	0.59±0.05	0.60±0.01
60:40 RBO:PS	ND	0.80±0.01	38.89±0.15	3.48±0.01	34.68±0.26	ND	23.72±0.09	0.69±0.01	0.78±0.02
70:30 RBOSL:PS	22.14±0.33	0.50±0.01	22.84±0.10	2.28±0.01	28.19±0.11	ND	22.84±0.11	0.65±0.04	0.62±0.01

RBOSL= rice bran oil structured lipid, RBO = rice bran oil, PS = palm stearin, CS = commercial shortening. ND = not detected

Table 6.1. Continued

Shortening blend	Fatty acid								
	8:0	14:0	16:0	18:0	18:1c	18:1t	18:2c	18:3	20:0
70:30 RBO:PS	ND	0.73±0.04	30.72±0.13	3.05±0.06	35.94±0.01	ND	27.97±0.04	0.95±0.21	0.81±0.01
CS #1	ND	1.12±0.01	45.26±0.02	4.66±0.01	38.20±0.01	ND	10.23±0.01	0.21±0.04	0.45±0.06
CS #2	0.42±0.03	0.19±0.01	14.07±0.06	12.30±0.11	28.53±0.09	11.42±0.24	30.03±0.11	2.64±0.01	0.63±0.33
PS	1.55±0.02	1.28±0.01	58.75±0.28	5.15±0.02	25.52±0.16	ND	7.10±0.03	0.46±0.05	0.28±0.18
RBO	ND	0.43±0.01	19.12±0.11	2.26±0.02	40.61±0.16	ND	35.56±0.11	1.06±0.02	0.99±0.01
RBOSL	28.89±0.04	0.23±0.04	9.88±0.01	1.25±0.01	29.61±0.03	ND	28.70±0.01	0.75±0.06	0.75±0.06

RBOSL= rice bran oil structured lipid, RBO = rice bran oil, PS = palm stearin, CS = commercial shortening. ND = not detected

Fig. 6.1 Solid fat contents of shortening blends of RBO or RBOSL with palm stearin and commercial shortenings. SP = RBOSL: palm stearin, RP = RBO: palm stearin, CS = commercial shortening, PS = palm stearin. The rectangle outlined by a dotted line indicates desired solid fat content range for a high stability shortening.

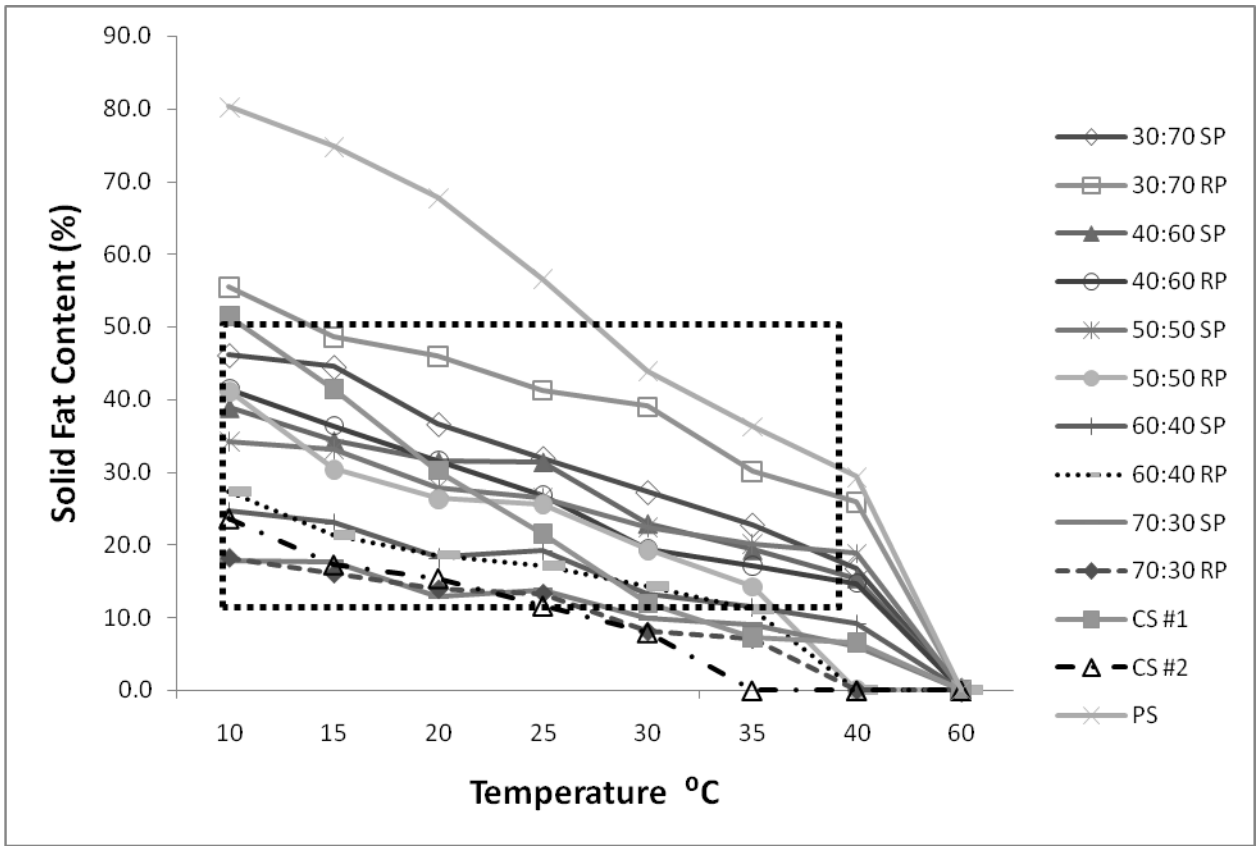
Solid Fat Content (%)
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
10
15
20
25
30
35
40
60
Temperature °C
30:70 SP
30:70 RP
40:60 SP
40:60 RP
50:50 SP
50:50 RP
60:40 SP
60:40 RP
70:30 SP
70:30 RP
CS #1
CS #2
PS

Fig 6.1

Fig 6.2 Melting profiles of shortening blends of rice bran oil (RBO) or rice bran oil structured lipid (RBOSL) with palm stearin (PS) and commercial shortenings (CS).

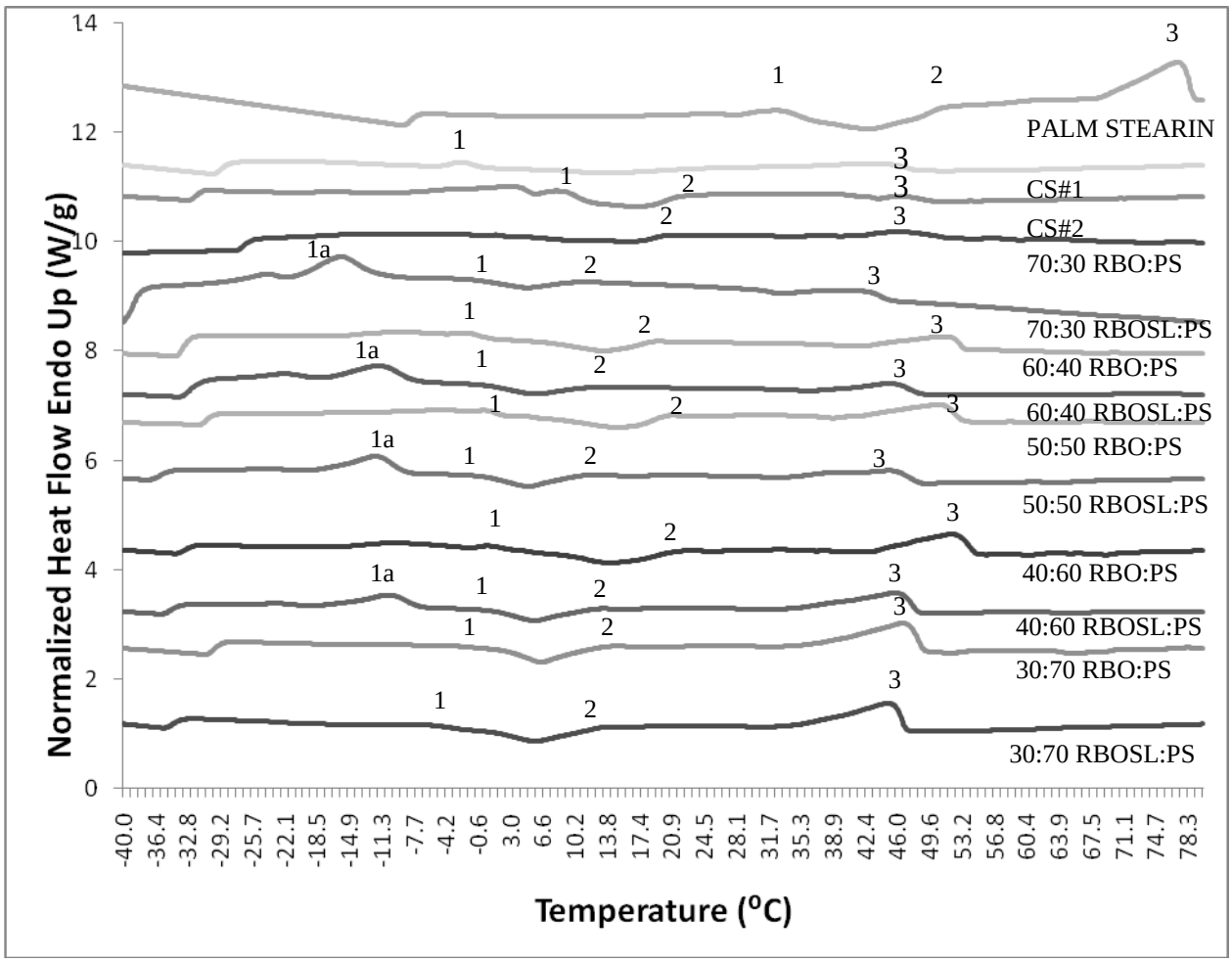
Normalized Heat Flow Endo Up (W/g)
Temperature (°C)
PALM STEARIN
CS#1
CS#2
70:30 RBO:PS
70:30 RBOSL:PS
60:40 RBO:PS
60:40 RBOSL:PS
50:50 RBO:PS
50:50 RBOSL:PS
40:60 RBO:PS
40:60 RBOSL:PS
30:70 RBO:PS
30:70 RBOSL:PS

Fig 6.2

Fig 6.3 Crystallization profiles of shortening blends of rice bran oil (RBO) or rice bran oil structured lipid (RBOSL) with palm stearin (PS) and commercial shortenings (CS)

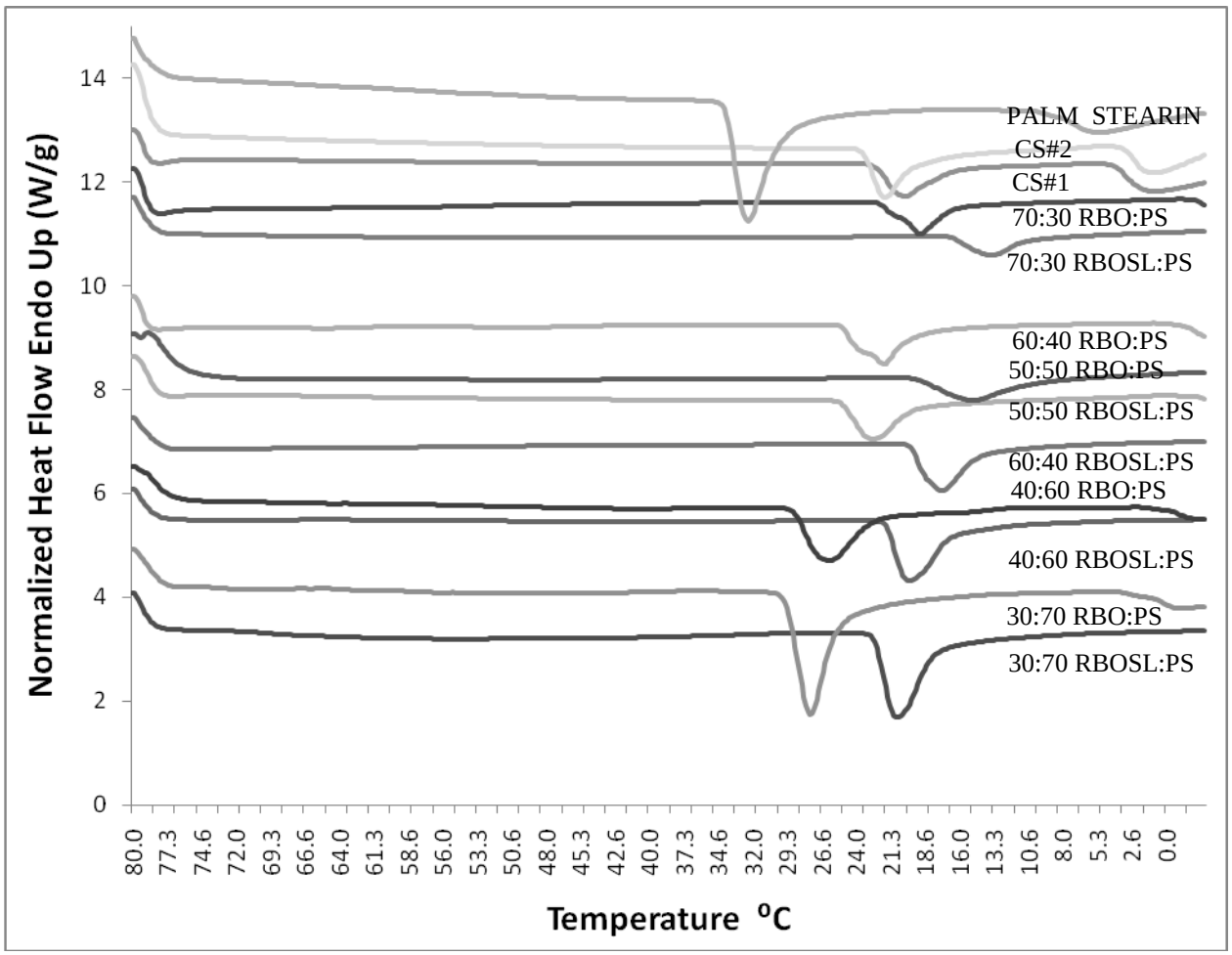
Normalized Heat Flow Endo Up (W/g)
14
12
10
8
6
4
2
0
80.0
77.3
74.6
72.0
69.3
66.6
64.0
61.3
58.6
56.0
53.3
50.6
48.0
45.3
42.6
40.0
37.3
34.6
32.0
29.3
26.6
24.0
21.3
18.6
16.0
13.3
10.6
8.0
5.3
2.6
0.0
Temperature °C
PALM STEARIN
CS#2
CS#1
70:30 RBO:PS
70:30 RBOSL:PS
60:40 RBO:PS
50:50 RBO:PS
50:50 RBOSL:PS
60:40 RBOSL:PS
40:60 RBO:PS
40:60 RBOSL:PS
30:70 RBO:PS
30:70 RBOSL:PS

Fig 6.3

CHAPTER 7

CONCLUSIONS

Rice bran oil was enzymatically modified to contain caprylic acid. The vitamin E content was significantly lower in RBOSL than in unmodified RBO because of the removal of tocopherols during short-path distillation. Differences in γ-oryzanol concentration before and after enzymatic modification were not significant. OSI values for CA and CA/RE were not significantly different from TBHQ. Peroxide values for CA and CA/RE were significantly lower than RBOSL without added antioxidant but not statistically different from TBHQ at days 9, 15, and 18. Mean *p*-anisidine values for CA 500 ppm on days 6, 9, 12 and 15 were significantly lower than RBOSL without added antioxidant and were comparable to TBHQ 500 ppm. The natural antioxidants, CA and CA/RE, were therefore shown to be as effective as TBHQ in increasing the oxidative stability of RBOSL. The effectiveness of combinations of natural and synthetic antioxidants such as CA/TBHQ is also notable and can provide an alternative that will allow for lower amounts of synthetic antioxidants to be used. The possible carcinogenic effect of synthetic antioxidant TBHQ and consumer demand for natural healthier products make natural antioxidants more desirable. Natural antioxidants are more accepted by consumers and health officials, can be labeled as flavorings, and may have positive effects on sensory properties. Our results indicate that natural antioxidants are viable alternatives to the synthetic antioxidant TBHQ for increasing the oxidative stability of a rice bran oil-based structured lipid.

Chemical and physical characteristics of a structured lipid containing rice bran oil and caprylic acid were determined. The differences in the chemical and physical properties of RBOSL were significantly different from those of RBO in most assays performed. This

characterization study can aid in determining future food applications of RBOSL. RBOSL may offer health benefits in addition to the health benefits of unmodified RBO.

The fatty acid profile of RBO compared to RBOSL after frying was almost unchanged. The color of sweet potato chips after frying in RBOSL was not significantly different from those fried in RBO. γ-Oryzanol concentration of RBOSL after frying was not significantly different from RBO. Free fatty acid content and *p*-anisidine values were significantly higher in RBOSL than RBO after frying. Oil uptake was significantly lower in RBOSL than in RBO. Results of triangle tests indicated that there was no significant difference in sweet potato chips but a significant difference in energy bars prepared with RBOSL compared to RBO. Consumer panels indicated a willingness to purchase both sweet potato chips and energy bars but greater standardization and changes in formulation may improve acceptability. These consumer panel results were very encouraging and indicate that products prepared with SLs with improved nutritional or health promoting properties may have viable commercial potential.

RBOSL blended with PS showed properties comparable to RBO:PS blends when the fatty acid profiles, solid fat content, melting and crystallization profiles and crystal forms were compared. Blending of RBOSL with PS is an option for producing *trans*-free shortening without hydrogenation and with the added health benefit of MCFA contained in RBOSL.