



- (51) International Patent Classification:
C08L 91/06 (2006.01) C11B 1/10 (2006.01)
- (21) International Application Number:
PCT/US2014/043385
- (22) International Filing Date:
20 June 2014 (20.06.2014)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
13/927,375 26 June 2013 (26.06.2013) US
- (71) Applicant: THE UNITED STATES OF AMERICA, AS REPRESENTED BY THE SECRETARY OF AGRICULTURE [US/US]; Washington, District of Columbia 20250 (US).
- (72) Inventor: BAKOTA, Erica L.; 200 Campanile Drive, East Peoria, Illinois 61611 (US).
- (74) Agent: GOLDBERG, Joshua B.; Nath, Goldberg & Meyer, 112 S. West Street, Alexandria, Virginia 22314 (US).
- (81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY,

BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

- (84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

- with international search report (Art. 21(3))
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))

- (54) Title: SPREADABLE PRODUCTS DERIVED FROM RICE BRAN OIL

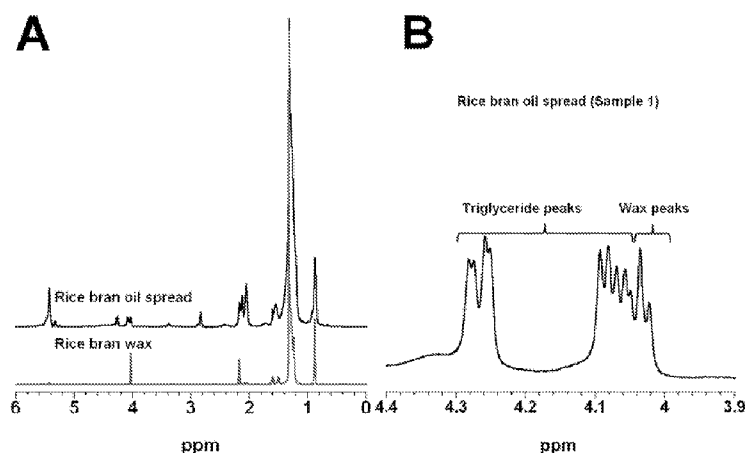


Figure 1

(57) Abstract: Products which are effective as a food or food ingredient may be prepared by acetone fractionation of rice bran oil. The products are spreadable, resembling a nut butter in appearance and texture, and are shelf-stable, resistant to oxidation, allergen-free and provide health benefits to consumers. The products are prepared by contacting rice bran oil with acetone to form a first oil/acetone mixture under conditions to establish a temperature of the mixture between -15 to -30°C, with the acetone being provided in an amount effective to extract the wax, some triacylglycerols, and phytosterols from the oil as a solid phase precipitate. This solid phase precipitate may then be recovered from the mixture and retained. The acetone fractionation of the rice bran oil at these low temperatures produces a solid phase product that is significantly enriched with rice bran wax, phytosterols and some fatty acids.

SPREADABLE PRODUCTS DERIVED FROM RICE BRAN OIL

Background of the Invention

Field of the Invention

[0001] This invention relates to novel spreadable food products obtained from rice bran oil which are rich in phytosterols, tocopherols, tocotrienols and γ -oryzanol, and methods for their production.

Description of the Prior Art

[0002] Rice bran oil has many properties that contribute to its appeal as a functional food ingredient. Rice bran oil contains approximately 85% triacylglycerols, with the remaining fraction consisting of waxes, mono- and diglycerides, free fatty acids, and other compounds such as tocopherols, tocotrienols, and phytosterols [Hemavathy & Prabhakar: Lipid-Composition of Rice (*Oryza-Sativa-L*) Bran, J. Am. Oil Chem. Soc. 1987, 64:1016-1019; Hu et al.: Comparison of isopropanol and hexane for extraction of vitamin E and oryzanols from stabilized rice bran, J. Am. Oil Chem. Soc. 1996, 73:1653-1656; Nagendra Prasad et al.: Health Benefits of Rice Bran - A Review, J. Nutr. Food Sci. 2011, 01]. The particular mixture of steryl ferulates found in rice is called γ -oryzanol and is also present at high levels in rice bran oil [Miller & Engel: Content of γ -Oryzanol and Composition of Steryl Ferulates in Brown Rice (*Oryza sativa* L.) of European Origin, J. Agric. Food Chem. 2006, 54:8127-8133; Juliano et al.: Antioxidant activity of gamma-oryzanol:

mechanism of action and its effect on oxidative stability of pharmaceutical oils, *Int. J. Pharm.* 2005, 299:146-154].

Compared with other vegetable oils, rice bran oil contains high concentrations of γ -oryzanol, tocopherols, and tocotrienols, all of which offer health benefits to consumers. γ -oryzanol has been shown to have antioxidant activity (Miller & Engel, *ibid*; Juliano et al., *ibid*; Gertz et al.: Testing and comparing oxidative stability of vegetable oils and fats at frying temperature, *Eur. J. Lipid Sci. Technol.* 2000, 102:543-551) as well as lower cholesterol in both rodents and humans (Rong et al.: Oryzanol decreases cholesterol absorption and aortic fatty streaks in hamsters, *Lipids.* 1997, 32:303-309; Yokoyama: Plasma LDL cholesterol lowering by plant phytosterols in a hamster model, *Trends Food Sci. Technol.* 2004, 15:528-531; Nakayama et al.: Comparative Effects of Two Forms of γ -Oryzanol in Different Sterol Compositions on Hyperlipidemia Induced by Cholesterol Diet in Rats, *Jpn. J. Pharmacol.* 1987, 44:135-143; Berger et al.: Similar cholesterol-lowering properties of rice bran oil, with varied gamma-oryzanol, in mildly hypercholesterolemic men, *Eur J Nutr.* 2005, 44:163-173). Tocopherols are potent antioxidants in food (Warner & Moser: Frying Stability of Purified Mid-Oleic Sunflower Oil Triacylglycerols with Added Pure Tocopherols and Tocopherol Mixtures, *J. Am. Oil Chem. Soc.* 2009, 86:1199-1207; Warner et al.: Oxidative Stability of Crude Mid-Oleic Sunflower Oils from Seeds with High γ - and δ -Tocopherol Levels, *J. Am. Oil Chem. Soc.* 2008, 85:529-533; Sherwin: Antioxidants for Vegetable Oils, *J. Am. Oil Chem. Soc.* 1976, 53:430-436), while tocotrienols have been shown to have neuroprotective, hypocholesterolemic, and anticancer properties (Sen et al.: Tocotrienols: Vitamin E beyond tocopherols, *Life Sci.* 2006, 78:2088-2098; Sen et al.: Tocotrienols in health and

disease: the other half of the natural vitamin E family, Mol Aspects Med. 2007, 28:692-728). Overall, rice bran oil is able to improve the blood lipid profile of rats when fed as part of the normal diet [16].

[0003] The same compounds thought to confer health benefits also contribute to enhanced resistance to oxidative degradation in the oil itself during heating. Crude rice bran oil has an induction time four to five times that of refined soybean oil in oxidative stability tests. As a result of both health and stability factors, the consumption of rice bran oil and its byproducts have increased dramatically in recent years (Chopra et al.: Structured lipids from rice bran oil and stearic acid using immobilized lipase from *Rhizomucor miehei*, Eur. J. Lipid Sci. Technol. 2008, 110:32-39).

[0004] Rice bran wax is one such byproduct and is obtained during the extraction of rice bran oil from rice bran. This wax is generally removed in subsequent rice bran oil processing steps. Rice bran wax, which consists primarily of even-numbered aliphatic waxy esters containing 44-64 carbons, is known to be capable of forming an organogel even at wax concentrations as low as 0.5 wt% (Marangoni: Organogels: An Alternative Edible Oil-Structuring Method, J. Am. Oil Chem. Soc. 2012, 89:749-780; Dassanayake et al.: Physical Properties of Rice Bran Wax in Bulk and Organogels, J. Am. Oil Chem. Soc. 2009, 86:1163-1173; Hwang et al.: Organogel Formation of Soybean Oil with Waxes, J. Am. Oil Chem. Soc. 2011, 89:639-647). These esters consist of C22 and C24 fatty acids linked to C24 to C40 aliphatic alcohols, with C24 and C30 being the predominant fatty acid and fatty alcohol species, although there is some variation in the alcohols (Vali et al.: A process for the preparation of food-grade rice bran wax and the determination of its composition, J.

Am. Oil Chem. Soc. 2004, 82:57-64). Rice bran wax has a melting temperature of 80-83°C, which is intermediate to that of two widely utilized edible waxes, carnauba wax and candelilla wax, and can form an organogel in vegetable oil at lower wax concentrations than either of these two waxes (Marongoni, *ibid*; Daddanayake *et al.*, *ibid*; Hwang *et al.*, *ibid*; Vali *et al.*, *ibid*). The recent demand for trans-fat free products has driven research into organogels and other structured lipids to replace partially hydrogenated oils. As consumer awareness and demand for trans-fat free products increases, so too will the value of ingredients that are capable of structuring lipids (Chopra *et al.*, *ibid*).

[0005] Peanut and tree nut allergies have also become more prevalent over the past two decades (Sicherer *et al.*: US prevalence of self-reported peanut, tree nut, and sesame allergy: 11-year follow-up, *J. Allergy Clin. Immunol.* 2010, 125:1322-1326). It is estimated that at least 1.5 million Americans suffer from peanut allergies (Lima & Guraya: Optimization Analysis of Sunflower Butter, *J. Food Sci.* 2005, 70:S365-S370). The severity of these allergies often requires that allergy sufferers avoid these ingredients completely. In the United States alone, it is estimated that food allergies cause approximately 2,000 hospitalizations and up to 200 deaths per year (Sampson: Anaphylaxis and Emergency Treatment, *Pediatrics.* 2003, 111:1601-1608). Meanwhile, nut-based spreads such as peanut butter and hazelnut butter have become ubiquitous in western cultures. Peanut butter is ranked as one of the most desired foods in consumers aged 20 years and younger (Lima & Guraya, *ibid*) and is ranked as the fifth most popular flavor in U.S. food products overall (Jeradechachai: New Product Opportunities Using One of America's Favorite Flavors, in: *Food*

Product Design Partner Series Ed. F. P. Design, Virgo Publishing, LLC 2012). New alternatives to nut spreads are needed to address the dearth of products available to those with nut allergies.

[0006] While hexane is the primary solvent used to extract oil from rice bran (Nagendra Prasad et al., *ibid*; Johnson & Lusas: Comparison of alternative solvents for oils extraction, *J. Am. Oil Chem. Soc.* 1983, 60:229-242), a multitude of extraction techniques have been developed for this purpose (Lerma-García et al.: Composition, industrial processing and applications of rice bran γ -oryzanol, *Food Chem.* 2009, 115:389-404). These procedures utilize a wide variety of solvents, including methanol, ethanol, isopropanol, dichloromethane, d-limonene and supercritical carbon dioxide (Hu et al., *ibid*; Hu et al., *ibid*; Meinke et al.: Solvent extraction of rice bran. Production of B-vitamin concentrate and oil by isopropanol extraction, *J. Am. Oil Chem. Soc.* 1949, 26:532-534; Mamidipally & Liu: First approach on rice bran oil extraction using limonene, *Eur. J. Lipid Sci. Technol.* 2004, 106:122-125). However, the procedures developed for extraction of crude rice bran oil from rice bran solids are intended to produce an oil; these methods differ from solvent fractionation and do not yield a spreadable product.

[0007] Thus, despite these and other advances, the need remains for improved spreadable food products suitable as peanut butter or nut butter substitutes, as fat replacements in baked products or as additives to existing fats/lipids.

Summary of the Invention

[0008] I have now invented novel products derived from rice bran oil which are effective as a food or food ingredient. The

products are spreadable, resembling a nut butter in appearance and texture, and are shelf-stable, resistant to oxidation, allergen-free and provide health benefits to consumers. The products are prepared by solvent fractionation of rice bran oil, wherein the rice bran oil is contacted with acetone to form a first oil/acetone mixture under conditions to establish a temperature of the mixture between -15 to -30°C, with the acetone being provided in an amount effective to extract the wax and phytosterols from the oil, as well as some triacylglycerols, as a solid phase precipitate. This solid phase precipitate may then be recovered from the mixture and retained. The acetone fractionation of the rice bran oil at the low temperatures described herein produces a solid phase product that is significantly enriched with rice bran wax, phytosterols and some fatty acids. Relative to the starting rice bran oil, the solid phase product contains increased proportions of wax and phytosterols, and also contains a significant proportion of tocopherols, tocotrienols and γ -oryzanol from the oil. The high content of phytosterols in the solid phase products, coupled with their physical properties and rheology, render these products suitable for a wide-range of food and nutritional applications.

[0009] In accordance with this discovery, it is an object of this invention to provide spreadable products from rice bran oil which are suitable for use as a food or food ingredient.

[0010] Another object of this invention is to provide spreadable products from rice bran oil which is free of allergens present in many nut-based spreads and thus may be used as a substitute for peanut butter or nut butter.

[0011] A further object of this invention is to provide spreadable products from rice bran oil which are free of trans-fats and may be used as a fat replacement or substitute for shortenings or other partially-hydrogenated oils.

[0012] Yet another object of this invention is to provide spreadable products from rice bran oil which are high in phytosterols, tocopherols, tocotrienols and γ -oryzanol, and thus provide positive health benefits to consumers.

[0013] Still another object of this invention is to provide spreadable products from rice bran oil which are rich in γ -oryzanol, an antioxidant, and thus can be used as an additive to fats and lipids to extend shelf life and fry life.

[0014] Other objects and advantages of this invention will become readily apparent from the ensuing description.

Brief Description of the Drawings

[0015] Figure 1A shows the NMR of rice bran oil spreads and rice bran wax as described in Example 1. Figure 1B shows the NMR of the rice bran spreads of sample 1. Although diagnostic of triacylglycerols and those of wax are close occur in the same region, they can still be differentiated for integration.

[0016] Figure 2 shows the DSC thermograms of rice bran oil spreads of Example 1.

[0017] Figure 3 shows the strain sweeps of rice bran oil spreads produced in Example 1. A) sample 1, 30 minutes at 4°C; B) sample 2, 60 minutes at 4°C; C) sample 3, 30 minutes at -20°C;

D) sample 4, 60 minutes at -20°C . Circles represent the elastic modulus, G' , and squares represent the loss modulus, G'' .

[0018] Figure 4 shows the oxidative stability indices of soybean oil with varying concentrations of rice bran oil spread as an additive as described in Example 1. The control sample is refined, bleached, deodorized soybean oil.

[0019] Figure 5 shows the canonical discriminant analysis as described in Example 1. Given a nominal classification variable and several independent variables, canonical discriminant analysis derives canonical variables (linear combinations of the independent variables) that summarize between-class variation. In this case, the canonical variables derived from the discriminant analysis show that spreadable rice bran products may be divided into four distinct groups.

[0020] Figure 6 shows the total polar compounds of soybean oil with added rice bran oil spread at varying concentrations as described in Example 2.

[0021] Figures 7a and 7b show the TAG (7a) and dimer (7b) concentrations in soybean oils with added rice bran oil spread as described in Example 2. All concentrations are expressed in area percent.

[0022] Figure 8 shows the oxidative stability of soybean oil-rice bran oil spread mixtures as described in Example 2.

[0023] Figure 9 shows the correct responses from discrimination testing of frying oils with added rice bran oil spread as described in Example 2. The dotted lines indicate the number of correct responses required to reach statistical

significance with $P \leq 0.05$. Superscripts indicate which threshold value is required.

[0024] Figure 10 shows the correct responses from discrimination testing of white bread and granola with rice bran oil spreads as a butter substitute as described in Example 2. Dotted lines indicate the number of correct responses needed to reach $P \leq 0.05$. Superscripts indicate which threshold value is required.

[0025] Figure 11 shows the acceptability ratings for baked goods incorporating rice bran oil spread as a butter replacement as described in Example 2.

Detailed Description of the Invention

[0026] The outstanding stability and positive health attributes of rice bran oil make it ideal for use as a starting material in the formulation of spreadable products, while the properties of rice bran wax lend promise to a structured lipid product based on rice bran. Rice bran oil, including crude rice bran oil preferred for use herein, may be obtained from commercial sources or extracted from rice bran (which typically includes the germ and inner husk of rice) using any conventional techniques. Suitable conventional techniques for extraction of crude rice bran oil include, but are not limited to, mechanical separation or chemical extraction, such as with hexane, supercritical carbon dioxide, petroleum ether, methanol, ethanol, isopropanol, dichloromethane or d-limonene. In the conventional processing of rice bran oil, the crude oil extracted from the bran is subsequently refined to remove gums, waxes, free fatty acids and pigments, and such refining typically includes one or

more steps of degumming, dewaxing, neutralization, bleaching and deodorization. However, because this refining reduces or removes the desired waxes and may reduce the concentration of other desirable components, the spreadable product of this invention is preferably prepared from rice bran oil which has not been refined or otherwise treated to remove the waxes therefrom (dewaxed). Moreover, it is also preferred to use non-hydrogenated oil. Thus, use of crude rice bran oil is preferred.

[0027] The rice bran oil starting material is contacted with acetone under conditions effective to form a homogeneous oil/acetone mixture and establish or provide a temperature of the mixture between -15 to -30°C, preferably between -15 to -28°C, and more preferably between -20 to -25°C. No additional solvent is required. Under these conditions, the wax and phytosterols in the oil, as well as some triacylglycerols, particularly those comprising myristic acid (C14:0), palmitic acid (C16:0), will relatively quickly precipitate as a solid phase. The initial temperatures of the acetone and oil are not critical, and thus pre-cooling is optional. However, pre-cooling of the acetone may facilitate lowering the temperature of the oil/acetone mixture within the above-mentioned contact temperature range. Establishment of the contact temperature may also be effected by cooling the oil/acetone mixture, such as by refrigeration. In a preferred embodiment, the temperature of the mixture is maintained or controlled within above-mentioned contact temperature range for a period of time effective to achieve the desired degree of precipitation of rice bran waxes, phytosterols, and a portion of the triacylglycerols. I have found that the duration of the time period during which the temperature of the mixture is maintained at this temperature

range will significantly affect the composition and rheological properties of the solid phase precipitate. When the oil in the mixture is cooled, the harder fats and waxes will precipitate first, yielding a hard solid phase product which is difficult to spread. Conversely, increasing amounts of the triacylglycerols (particularly those comprising unsaturated C18 fatty acids) will precipitate with time, yielding a progressively softer solid phase material. Thus, the temperature of the mixture is preferably maintained within above-mentioned contact temperature range for a period of time until the solid phase precipitate comprises a wax to total triacylglycerol molar ratio between 1:4 and 2:3 (i.e., the molar proportion of wax to triacylglycerol is between 0.2:0.8 to 0.4:0.6). In a particularly preferred embodiment, the temperature of the mixture is maintained within above-mentioned contact temperature range for a period of time until the solid phase precipitate comprises a wax to total triacylglycerol molar ratio between 1:3 and 2:3 (the molar proportion of wax to triacylglycerol is between 0.25:0.75 and 0.4:0.6). The determination of the molar proportions may be performed using techniques known in the art and as described in Example 1. As a practical matter, the precise period of time of the contact will vary with the desired softness of the solid phase product, absolute and relative amounts of acetone and oil, temperature, and the type of contact equipment as described below, and may be readily determined empirically by the skilled user. However, by way of illustration and without being limited thereto, preferred times are typically between approximately 20 to 60 minutes.

[0028] To contact the oil, the acetone is provided in an amount effective to extract (precipitate) the wax and phytosterols from the oil as a solid phase precipitate at the

contact temperature range referred to above. Some triacylglycerols, particularly those comprising myristic acid (C14:0), palmitic acid (C16:0) will be precipitated as well. Although the amount of acetone is variable, increasing the amount facilitates separation and recovery of the solid phase precipitate from the oil/acetone mixture. Thus, without being limited thereto, in a preferred embodiment the amount of acetone is greater than or equal to 1 ml of acetone per gram of oil, more preferably greater than or equal to 1.5 ml of acetone per gram of oil, yet more preferably greater than or equal to 2 ml of acetone per gram of oil, and most preferably greater than or equal to 2.5 ml of acetone per gram of oil. Use of acetone in amounts less than 1 ml per gram of oil produces an inferior product and greatly prolongs filtration times for recovery of the solid phase product.

[0029] Contact of the acetone and oil, may be conducted in a variety of conventional liquid/liquid contactor devices. A number of liquid/liquid contact devices have been described for other liquid/liquid extractions and are suitable for use herein, and include mixed tanks (or mixer-settlers), columns, mechanically assisted gravity devices and centrifugal extractors, with mixed tanks being preferred.

[0030] Upon completion of the contacting, the resultant solid phase precipitate is separated from the remaining oil/acetone mixture. In the preferred embodiment, the solid phase precipitate is may separated or removed, for example, by conventional techniques such as by settling, centrifugation, decantation or filtration, with filtration being preferred. The solid phase precipitate may be recovered and optionally further treated, such as by drying.

[0031] The solid phase product recovered from the extraction is a composition of concentrated rice bran wax, phytosterols, and a portion of the triacylglycerols present in the oil, particularly those comprising saturated fatty acids, more particularly myristic acid (14:0), palmitic acid (16:0), behenic acid (22:0) and lignoceric acid (24:0). As noted above, the extraction of the rice bran oil at the low temperatures described herein produces a solid phase product that is significantly enriched with these components. Relative to the starting rice bran oil, the solid phase product contains increased proportions of wax, phytosterols, and triacylglycerols comprising myristic acid (14:0), palmitic acid (16:0), behenic acid (22:0) and lignoceric acid (24:0). The solid phase product also contains a significant proportion of tocopherols, tocotrienols and γ -oryzanol from the oil. The solid phase precipitate preferably comprises a wax to triacylglycerol molar ratio between 1:4 and 2:3, particularly between 1:3 and 2:3.

[0032] The precipitated, solid phase products prepared herein are suitable for use in a wide-variety of food applications. By way of example and without being limited thereto, the products may be used as spreads, fat replacements or substitutes or as fat/lipid additives. In a first preferred embodiment, because the solid phase product is derived from rice bran oil and is free of allergens associated with many nut-based spreads, the product may be used as a substitute for peanut butter or nut butter. In another embodiment, the products may be used as a trans-fat-free fat replacement or substitute, such as for shortenings or other partially-hydrogenated oils, particularly for use in baked goods. In yet another embodiment, because the product is high in antioxidants, the product may be used as an additive to fats and lipids to extend their shelf life and fry

life. In any of these or other embodiments, the high levels of phytosterols, tocopherols, tocotrienols and γ -oryzanol in the products also provide positive health benefits to consumers.

[0033] The following examples are intended only to further illustrate the invention and are not intended to limit the scope of the invention which is defined by the claims.

Example 1

[0034] In this example, the affect of solvent fractionation conditions on a spreadable rice bran oil product were examined. Crude rice bran oil was mixed with cold acetone at two different temperatures and allowed to incubate for two different intervals to investigate the effects of temperature and time on the chemical composition.

Materials and Methods

Materials

[0035] Hexane-extracted, alkali-refined, bleached and deodorized soybean oil (SBO) was obtained from a commercial processor. Crude rice bran oil (RBO) was obtained from Riceland Foods (Stuttgart, AR). Oils were stored at -20°C prior to use. Tocopherols (97-98% pure, as determined by GC) were purchased from Matreya (Pleasant Gap, PA). Tocotrienols (~95.5% pure, as determined by GC) were from ChromaDex (Irvine, CA). Oryzanol was obtained from Spectrum Organics (New Brunswick, NJ). HPLC grade hexane and tetrahydrofuran were obtained from Fisher Scientific (Fair Lawn, NJ). 5 α -cholestane, sitosterol, sitostanol, and stigmasterol were from Sigma-Aldrich, Inc. (St. Louis, MO). Campesterol was purchased from Steraloids (Newport,

RI). Each phytosterol standard was 95-97% purity, which was verified by GC. N,O-Bis(trimethylsilyl)fluoroacetamide with 1% trimethylchlorosilane (BSTFA+1% TMCS) was purchased from Supelco (Bellefonte, PA). Commercial peanut butter was obtained from a local grocery store (Kroger Company, Cincinnati, OH). All other chemicals and solvents were obtained from Sigma-Aldrich (St. Louis, MO), unless otherwise stated, and were of ACS grade or HPLC grade for solvents.

Solvent fractionation to produce spreadable products from rice bran oil

[0036] Rice bran oil was brought to room temperature prior to fractionation. Acetone was chilled in a standard refrigerator or freezer at either 4°C or -20°C, respectively, for several hours prior to fractionation in a capped Erlenmeyer flask. 80 g rice bran oil was added to a beaker; 200 mL cold acetone was slowly added, and the mixture was mixed briefly on a stirplate before being held at 4°C or -20°C for either 30 or 60 minutes (Table 1). The mixture was then filtered through Whatman No. 1 filter paper using a Buchner funnel. The filter paper was scraped periodically to harvest the product, which was dried under vacuum for 48 hours and then weighed. The filtered oil was discarded. Solid fat contents of the cold slurries were measured before filtration and then again on the final products. Each fractionation condition was replicated a minimum of four times. Yields varied but were typically less than 10% of the mass of the starting oil. Yields were based on the average of 3 replicates (Table 6).

Determination of free fatty acids (FFA) in crude rice bran oil

[0037] FFA concentrations of crude rice bran oil were measure according to AOCS Official Method Ca 5a-40 (AOCS: *Official methods and recommended practices of the American Oil Chemists' Society*, 5th edn. AOCS Press, Champaign 1998). Measurements were taken in triplicate. Free fatty acids were calculated as oleic acid using the following formula:

$$\text{Free fatty acids as oleic, \%} = \frac{\text{mL alkali} \times 0.25 \times 28.2}{\text{mass, g of test portion}}$$

The FFA content of crude rice bran oil was 0.800%, which was determined to be acceptable for use.

Fatty acid composition of oils

[0038] Fatty acid methyl esters (FAME) were made according to the method published by Ichihara et al. (An improved method for rapid analysis of the fatty acids of glycerolipids, *Lipids*, 1996, 31:535-539). Briefly, 10-20 mg of oil was dissolved in 2 mL hexane. 0.2 mL methanolic KOH (2N) was added and the solution was vortexed for 2 min. The hexane portion was transferred to autosampler vials. Fatty acid compositions of each sample were determined by GC analysis on an Agilent 6890 GC (Palo Alto, CA) with a Supelco (Bellefonte, PA) SP-2380 capillary column (30m x 0.25 mm ID x 0.20 µm film). Helium was used as a carrier gas with a flow rate of 1 mL/min, the injector was held at 220°C, with a 50:1 split ratio, the oven was held at 135°C, and the FID was held at 220°C. Commercial FAME standards GLC 15a (Nu-Chek Prep, Elysian, MN), and Supelco 37 component FAME Mix were used to identify peaks and verify GC performance. Samples were measured in duplicate. Results are reported as % composition. Sterol and wax esters are not transesterified under these conditions and free fatty acids are not methylated; thus, fatty

acid composition reflects only the composition of glycerolipids present in the samples.

Tocopherol and tocotrienol analysis

[0039] Tocopherols and tocotrienols were analyzed by an HPLC system, which consisted of a Varian (Varian, Inc., Palo Alto, CA) Pro-Star pump, autosampler, and fluorescence detector. The mobile phase consisted of hexane:tetrahydrofuran (97:3 v/v, made fresh daily) pumped at 2 mL/min. Tocopherols were separated using an Inertsil (Varian, Inc.), silica column (5 μ m, 150 Å, 250 x 4.6 mm I.D.). The fluorescence detector was set with an excitation wavelength of 290 nm and emission wavelength of 330 nm. Data collection and integration were performed with Varian Star Chromatography Ver. 6.0. Tocopherol peaks were identified by retention time of known standards. Tocopherols and tocotrienols were quantified using external standard curves at concentrations ranging between 0.5 μ g/mL - 10 μ g/mL. A mixture of α -, β -, γ -, and δ -tocopherol/tocotrienol standards was injected periodically to verify HPLC performance.

Oryzanol analysis

[0040] Phytosterol ferulates were quantified by HPLC using a Thermo Separation Products HPLC (Thermo Scientific, San Jose, CA) connected by a Starrett flow splitter (90:10 UV/FL) to a Surveyor FL Plus fluorescence detector (Thermo Scientific, San Jose, CA) and SpectraSYSTEM UV2000 in parallel. Samples (20 μ L) were separated on a YMC-Pack-Diol-NP, 5 μ m, 4.6 X 250mm column (YMC, Wilmington, NC). The mobile phase consisted of a 97:3 mixture of hexane:tetrahydrofuran (Fisher Scientific, Fair Lawn, NJ) with a 2.0mL/min flow rate. Oryzanol eluted as a single peak and was detected by UV at 325 nm. Oryzanol was identified based upon retention time of a known standard (oryzanol, 98% pure, Spectrum Organics, New Brunswick, NJ) and quantified by

external calibration (1 μg /mL to 1 mg/mL). Data collection and integration were performed with ChromQuest 5.0.

Phytosterol Analysis

[0041] Crude rice bran oil (20 mg) and rice bran oil spreads (12–18 mg) were weighed into 13 x 100 mm screw cap glass tubes, and after the addition of 100 μg 5 α -cholestane (internal standard), were saponified with 2.5 N KOH in ethanol at 65°C for 1.5 hr, with periodic mixing by vortex to ensure complete saponification. After cooling to room temperature, unsaponifiable material (containing phytosterols) was extracted three times with hexane. Hexane fractions were combined and the solvent evaporated under a gentle stream of nitrogen. Phytosterols were converted to trimethylsilyl ethers after dissolving in equal volumes of pyridine and BSTFA+1% TMCS. Samples (1 μL) were injected in duplicate by autosampler onto a Varian (Palo Alto, CA) 3800 GC equipped with an FID and a DB-5 (Agilent, Santa Clara, CA) capillary column (30m x 0.25mm x 0.25 μm). Helium was used as a carrier gas at 1 mL/min, with a 1:50 injector split. Injector temperature was 270°C, and detector temperature was 280°C. The column oven initial temperature was 250°C for 0.5 min, increased at 10°C/min to 270°C and held for 30 min, then increased at 10°C/min to 280°C and held for 3.5 min. Data collection and peak integration were performed using Varian's Galaxie Chromatography Software version 1.9. Phytosterol peaks were identified by comparison of relative retention times with those of commercially available standards, and by comparison to literature values for relative retention times. Quantitation was carried out by the internal standard method developed with available standards. For phytosterols with no available commercial standard, the response factor for β -sitosterol was used for quantitation. The

identities of phytosterol peaks were confirmed by GC-MS analysis performed on an Agilent 6890 GC-MS equipped with a HP-5MS capillary column (30m x 0.25mm x 0.25µm), a 5973 mass selective detector, and an 7683 autosampler. The transfer line from GC to the MSD was set to 280°C. The injector and oven temperature programs were the same as described for the GC-FID instrument above. MSD parameters were as follows: scan mode, 50-600 amu, ionizing voltage, 70 eV, and EM voltage, 1823 V. Saponification and extraction of each sample was performed in duplicate.

Nuclear magnetic resonance (NMR)

[0042] ^1H NMR spectra were acquired with benzene- d_6 as solvent on a Bruker (Billerica, MA) Avance 500 spectrometer operating at 500 MHz. Chemical shifts are reported relative to the chloroform peak (7.16 ppm). The NMR tube was heated at 60 °C during measurement to dissolve all components. The triacylglycerol backbone peak at 4.06 – 4.18 ppm for two protons (quartet) and the wax ester peak at 3.96 – 4.06 ppm for two protons (triplet) were compared to obtain the molar ratio between triacylglycerol and wax ester. SpinWorks 3.1.7 software was used for integration of peak areas.

Dropping point

[0043] Dropping points were determined according to AOCS Standard Method Cc 18-80 (AOCS, *ibid*). The dropping point apparatus, a Mettler FP83HT Dropping Point Cell with FP90 Central Processor (Mettler Toledo, Columbus, OH), was calibrated using 0.5% powdered carbon in lauric acid. Samples were melted at 80°C and poured into clean pre-chilled sample cups. The filled sample cups were allowed to stand in the freezer (-20°C) for at least 15 minutes prior to measurement to allow the samples to re-solidify. Meanwhile, the dropping point furnace was heated to 50°C. The sample cup was removed from the freezer,

inserted into the sample holder, and immediately placed into the dropping point furnace. The sample holder was rotated to ensure proper positioning of the horizontal slits. Waiting time was fixed at 60 seconds, after which the temperature was ramped at 1°C/min until the dropping point was reached. The dropping point furnace was then immediately returned to the starting temperature. Samples were measured in triplicate.

Differential scanning calorimetry (DSC)

[0044] DSC was performed on a TA Instruments DSC, Model Q2000 with RCS cooling system (New Castle, DE). The baseline was calibrated with a sapphire disk. Samples weighed to 8 ± 1 mg were encapsulated in Tzero aluminum pans. The instrument was purged with dry nitrogen at 50 mL/min. Cooling and heating were done at 5°C/min at the temperature range of -60°C to 120°C. Results were analyzed using TA Universal Analysis software.

Solid Fat Content (SFC)

[0045] Measurements were made according the AOCS Official Method Cd 16b-93 (AOCS, *ibid*). Briefly, to standardize sample histories, NMR tubes containing either crude rice bran oil or rice bran oil-derived spreads were heated sequentially at 100°C for 15 minutes, 60°C for 5 minutes, and 0°C for 60 minutes. The tubes were then allowed to sit at room temperature for 30 minutes prior to analysis. All measurements were performed with a 20 MHz Bruker Minispec mq20, with a standard Daily Check performed within 24 hours prior to measurements. Bruker standards (0.0%, 31.6%, and 74.3% SFC) were used to perform the Daily Check. The method used was the solid fat content application, which was designed and installed on the mq20 by Bruker. After the 30 minute incubation time at room temperature, samples were read for approximately 7-10 seconds each, as prompted by the instrument. Each sample was analyzed

five times. Measurements were also performed on the cold slurries after the incubation periods described in Table 1. Because the standardization procedure described in AOCS Official Method Cd 16b-93 (AOCS, *ibid*) would evaporate the acetone and change the solids content of the cold slurries, SFC measurements on cold slurries were performed immediately following the prescribed incubation time with no further modifications. To minimize the impact of the 40°C magnet heating the sample during measurements, SFC measurements on cold slurries were performed only in triplicate, rather than five-fold.

Rheology

[0046] After an initial determination that the rice bran spreads were stable over the time periods required for measurement, the linear viscoelastic region was determined. Frequency sweeps were performed at 0.1 % strain, which was within the linear viscoelastic region for strain. Strain sweeps were performed at 1 rad/sec, which was within the linear viscoelastic region for frequency. Data were acquired at 10 points per decade using a TA Instruments ARES controlled strain rheometer with an 8 mm stainless steel parallel plate geometry. The gap was set at 1.5 mm. Measurements were performed in triplicate. Elastic and loss moduli were determined from strain sweeps by taking the mean of modulus values from 0.01 to 0.1% strain, which corresponded to one decade in the linear viscoelastic region.

Oxidative Stability Index (OSI)

[0047] The OSI at 110°C was determined according to AOCS Official Method Cd 12b-92 (AOCS, *ibid*). A Metrohm (Herisau, Switzerland) 743 Rancimat was used, which calculated OSI values based on induction time. All samples were measured in triplicate.

Statistical Analysis

[0048] A discriminate analysis was performed (JMP 10.0.0, 2010 SAS Institute, Inc.) to determine the separation between groups. Temperature and time were used as the independent variables, and fatty acid composition, tocopherols/tocotrienols composition, solid fat content, dropping point, and elastic modulus were used as the dependent variables. Only the first two replicates in each technique were considered for the discriminant analysis, as this was the number of replicates performed to acquire fatty acid compositional data. However, many of these techniques were executed with more replicates as stated above (and all replicates were considered in individual analyses). Based on these parameters, the following variables were selected as contributing to group separation: C14:0, C16:0, C18:0, C18:1-c9, C18:1-c11, C18:2, C18:3, α -tocotrienol, and β -tocopherol.

Results

Composition of rice bran oil spreads

[0049] We attempted to tune the solvent fractionation conditions in order to achieve a spreadable rice bran oil product. Fractionation was attempted with several solvents, as well as with no solvent at all. However, spreadable products were only obtained using acetone. Dry fractionation (no solvent) yielded a chilled rice bran oil that was too thick to be filtered effectively, with all samples warming up to room temperature before meaningful separation of fractions could take place. Fractionations of crude rice bran oil in cold acetone were performed at refrigeration temperature or freezer temperature for either 30 or 60 minutes. This resulted in the production of four distinct rice bran oil spreads (Table 1).

Different fractionation conditions led to differences in fatty acid composition and physical properties for each spread.

[0050] All four rice bran oil spread samples showed differences in chemical composition from the rice bran oil from which they were derived (Table 2). Compared with crude rice bran oil, fatty acid compositions of the four resulting spreads were relatively enriched with myristic (14:0) and palmitic (16:0) acids. Crude rice bran oil contained higher proportions of 18:0, 18:1, 18:2, 18:3, and 20:0 acids. Eicosenoic acid (20:1) was detected in all of the spread samples, whereas none was detected in the crude rice bran oil. Because the fractionation process concentrates some components of the mixture relative to others, eicosenoic acid may have been enriched to the extent that it exceeds the limit of detection only after isolation of the product. The spreads were also relatively rich in 22:0 and 24:0 acids, with the proportion of these fatty acids often two to threefold higher than that found in the crude rice bran oil. It is unclear why the rice bran oil spreads are enriched in some fatty acids while relatively depleted in others. Among the rice bran oil spreads, the compositions were very similar, but chemical and physical analyses yielded some differences. Longer incubation times (samples 2 and 4) resulted in higher concentrations of oleic, linoleic, and linolenic acid but lower proportions of myristic, palmitic, and eicosanoic acids. The lower temperature incubations (samples 3 and 4) also contained higher proportions of 22:0 and 24:0 than the samples obtained at refrigeration temperature. Fatty acid analysis did not measure levels of rice bran wax, which were determined by NMR. NMR analysis revealed sets of peaks that are consistent with triacylglycerols. Although peaks of many different types of compounds show some

overlap with triacylglycerol peaks, other sets of diagnostic peaks indicated a considerable amount of rice bran wax (Figure 1). The data suggest that the two principal components of the rice bran oil spreads are triacylglycerols and rice bran wax. Furthermore, the samples with the shortest incubation times (samples 1 and 3) contained the highest levels of rice bran wax and lowest levels of rice bran triacylglycerols, while the samples with the longest incubation times (samples 2 and 4) contained relatively lower proportions of rice bran wax but higher proportions of rice bran triacylglycerols. This is consistent with the observation via fatty acid analysis that samples 2 and 4 contained higher levels of oleic, linoleic, and linolenic acids. From these data, it is clear that longer incubation times result in the enrichment in C18 fatty acids in the final spread samples.

[0051] Similarly, tocopherols, tocotrienols, and oryzanol were found in lower levels in the spreads than in crude rice bran oil (Table 3). On average, the spreadable products contained 40-70% of the tocopherols, tocotrienols, and oryzanol concentrations of crude rice bran oil. Concentrations were highest in samples with 60-minute incubation times, compared with those samples derived from 30-minute incubations. Samples 2 and 4 were enriched in α -tocopherol, α -tocotrienol, β -tocopherol, γ -tocopherol, γ -tocotrienol, δ -tocotrienol, and oryzanol relative to samples 1 and 3. δ -tocopherol was the only analyte that was not correlated with incubation time.

[0052] Phytosterols, on the other hand, were enriched in the rice bran spreads compared with crude rice bran oil. Most sterols were present at concentrations ranging from 109% to 321% of those found in crude rice bran oil. As the sterols and stanols measured are solid at the temperatures used to carry out

the fractionation, it is not unexpected that the extracted products would contain higher levels of sterols and stanols. However, squalene, a steroid precursor, was also enriched in the spreads, although to a much higher degree. Squalene concentrations in the spreads were approximately 10-12 times higher than those found in the crude rice bran oil. Rice bran oil is known to be rich in unsaponifiable material and specifically, squalene (Ghosh: Review on Recent Trends in Rice Bran Oil Processing, J. Am. Oil Chem. Soc. 2007, 84:315-324). It may be that the hydrophobic, oily nature of squalene contributes to its disproportional enrichment in the spreads compared with other phytosterols.

[0053] Solid fat contents of the cold slurries varied from 0.79% to 1.24% (Table 5). The mean solids contents of the unfiltered slurries were not significantly different among samples, as determined by an unpaired T-test. However, the differences were more prevalent in the final spreadable products. The increased solid fat content (Table 5) in the samples with 30 minute incubation times (samples 1 and 3) is consistent with the higher levels of 14:0 and 16:0 fatty acids in these samples as determined by fatty acid analysis. Therefore, shorter incubation times favor the incorporation of triacylglycerols that are solid at room temperature. Solid fat content also provides information about wax concentrations in rice bran oil spreads. Solid fat content does not directly measure wax levels, but as a lipid that is solid at the temperature of SFC measurement, wax is included in SFC measurements. The higher levels of rice bran wax in samples 1 and 3 detected by NMR (Table 5) are consistent with higher solid fat contents in these samples (29.10% and 28.41%, respectively) compared with those of samples 2 and 4 (27.89% and 27.64%,

respectively). All four samples show a significantly higher ($P < 0.0001$) solid fat content than crude rice bran oil, which has a SFC of 1.96% at room temperature. The relative areas of the integrated triacylglycerol and wax ester peaks obtained via NMR were used to estimate the molar ratio of triacylglycerol to wax ester (Table 5). For all samples, the ratio of TAG:wax ester was approximately 2:1. As rice bran oil is typically approximately 85% triacylglycerols (Hemavathy & Prabhakar, *ibid*), it can be determined via NMR and SFC measurements that the fractionation process used to create rice bran oil spreads produces a significant enrichment in rice bran wax.

Thermal behavior of rice bran oil spreads

[0054] Differential scanning calorimetry was performed to determine the thermal transitions of compounds present in the rice bran spreads. The DSC shows two transitions: the first (T1) begins around -27°C and ends at approximately 7°C , and the second (T2) occurs at 70°C (Figure 2). The T1 transition corresponds to the melting of triacylglycerols, and the T2 transition represents the melting of rice bran wax. Crude rice bran wax does not have a sharp melting point but rather melts between 75°C and 79°C . Pure rice bran wax melts at approximately 80°C (Vali et al., *ibid*). As the rice bran wax is surrounded by rice bran oil in these samples, a T2 transition below 80°C is expected. Slightly higher temperatures for T2 in samples 1 and 3 as determined by DSC are consistent with the NMR and SFC data (Table 5), which indicate that wax levels are highest in these samples. Samples 2 and 4, which display the lower two T2 temperatures, are shown by NMR and SFC to have lower wax levels. The dropping point of all samples was determined to be between 63°C and 67°C (Table 5), with sample 1 showing the highest

dropping point and sample 4 showing the lowest. Interestingly, the dropping points did not show a correlation with incubation time, as has been observed with the other techniques. This could be due to minor compositional differences that were not identified here or the relatively low sensitivity of the dropping point technique.

Rheology of rice bran oil spreads

[0055] The products obtained from the solvent fractionation visually resembled a nut butter in both appearance and texture. Rheological tests were performed to further characterize the texture of the rice bran spreads. Frequency and strain sweeps (Figure 3) were performed to determine the elastic and loss moduli of the rice bran spreads (Table 5). All four spreads had higher elastic moduli than commercial peanut butter ($2.87 \cdot 10^4$ Pa). The samples derived from 30-minute incubations had the highest elastic moduli, while the samples derived from 60-minute incubations had the lowest. This is consistent with the higher levels of myristic acid, palmitic acid, and rice bran wax contained in the 30-minute samples, as all of these components are solid at room temperature, which would produce a more rigid spread. The lower elastic moduli in the 60-minute samples are also supported by the fatty acid analysis data, which suggest higher levels of oleic, linoleic, and linolenic acids (all of which are liquid at room temperature) in the 60-minute samples. Higher levels of liquid oil would produce a spread that is softer in texture, which is indeed what is observed by rheology.

Oxidative stability of rice bran oil spreads

[0056] Crude rice bran oil has an OSI of 37.24 hours, over four times longer than refined soybean oil. It is thought that the unusually high resistance of rice bran oil to oxidation is

imparted by γ -oryzanol (Lerma-García et al., *ibid*; Wang et al.: Antioxidant activity of phytosterols, oryzanol, and other phytosterol conjugates, J. Am. Oil Chem. Soc. 2002, 79:1201-1206). If this is indeed true, products such as spreads that are relatively rich in γ -oryzanol should show even better stability to lipid oxidation than crude rice bran oil. Unfortunately, the oxidative stability of the rice bran oil spreads was difficult to determine directly, as the rigid texture of the spreads did not allow air to be bubbled uniformly through the samples. This made oxidative stability determination by the traditional method nearly impossible for all samples except the crude rice bran oil. Instead, the rice bran oil spreads were dissolved at 1, 2, and 3 wt% in refined, bleached, and deodorized soybean oil and the oxidative stabilities of the mixtures were measured (Figure 4). Alone, soybean oil had an OSI of 8.44 hours. At 1 wt%, all rice bran oil spreads extended the OSI to approximately 12 hours, and at 2 wt% the OSI was extended to approximately 13 hours. It is expected that as the proportion of rice bran oil and/or rice bran oil spread in soybean oil is increased, the OSI of the mixture will continue to increase. Thus, the rice bran oil spreads impart some resistance to oxidation, an effect which is likely due to the presence of tocopherols, tocotrienols, and γ -oryzanol.

Discussion

[0057] Fractionation of crude rice bran oil with acetone yielded a spreadable product that is statistically significantly different from rice bran oil. For the spreadable rice bran oil products, a discriminant analysis was performed to further elucidate differences between groups. For a set of observations containing one or more quantitative variables and a

classification variable defining groups of observations, discriminant analysis classifies each observation into one of the groups (*SAS/STAT 9.22 User's Guide*. SAS Institute Inc., Cary, NC, USA 2008). The analysis was performed using fatty acid compositions, tocopherols and tocotrienols concentrations, solid fat content, dropping point, and elastic modulus as the quantitative variables, and temperature and time as classification variables. The analysis produced three canonical functions, which are linear combinations of dependent variables that explain the separation between groups. It was found that the first two canonical functions collectively explain over 98% of the group differences, which was statistically significant at $P < 0.0001$. Using these canonical functions, samples could be sorted into different experimental groups with no misclassifications. Figure 5 shows the four populations obtained by solving the first two canonical functions using the values of each independent variable. The value of the solved first canonical function produces the x coordinate and the value of the solved second canonical function produces the y coordinate. Figure 5 illustrates that the canonical functions produced by the discriminant analysis could be used, in conjunction with the measured values for each independent variable, to correctly classify samples as belonging to the correct temperature and time groups based. Therefore, it was determined that four different experimental conditions yielded four statistically distinct populations. All four populations were significantly different from crude rice bran oil, which could not be considered for discriminant analysis, as not all tests that could be performed on a spread could be performed on the oil.

[0058] The sample obtained at -20°C for 60 minutes (sample 4) was the most similar to rice bran oil in chemical composition, thermal behavior, and textural properties. The sample obtained at 4°C for 60 minutes (sample 2) showed larger differences from crude rice bran oil compared with sample 4. These two samples were statistically distinct from each other and from crude rice bran oil. The samples held for 30 minutes showed the furthest departure from rice bran oil in terms of physical properties, chemical composition, and rheology.

[0059] Crude rice bran oil is relatively rich in unsaturated fatty acids compared to the spreads, while the spreads are richer in palmitic and myristic acids. It is interesting that shorter, not longer, incubation times produced samples that differed most from the original oil in fatty acid composition. One possible reason for this is that longer incubation times allowed the crystallization of fats with lower melting points from the bulk rice bran oil, while shorter incubation times did not allow a sufficient decrease in temperature in the fractionation mixture to achieve crystallization of these fats. The resultant spreads end up being relatively rich in fatty acids that have high melting points (stearic and myristic acid) while being relatively poor in fatty acids with lower melting points (oleic, linoleic, and linolenic acid). This hypothesis is consistent with higher levels of wax in the samples with shorter incubation times, since wax has a higher melting point and would have been one of the first species to crystallize in this fractional crystallization. Higher levels of wax would then support the higher T₂ transition temperatures in these samples. The enrichment in phytosterols of rice bran oil spreads suggests that the spreads have the potential to impart

health benefits to consumers, although that has not been explored in these spreads to date.

Table 1. Solvent fractionation conditions for four rice bran oil spreads.

Sample	Rice Bran Oil [°C]	Acetone [°C]	Incubation [°C]	Time [minutes]
1	23	4	4	30
2	23	4	4	60
3	23	-20	-20	30
4	23	-20	-20	60

Table 2. Fatty acid compositions of rice bran oil spreads, reported as % composition.

Fatty Acid	Sample 1	Sample 2	Sample 3	Sample 4	Crude RBO
14:0	0.45	0.37	0.40	0.37	0.31
16:0	17.64	16.49	17.01	16.52	15.52
18:0	1.44	1.56	1.55	1.59	1.64
18:1 c9	40.26	40.73	40.48	40.74	42.30
18:1 c11	0.82	0.82	0.84	0.82	0.00
18:2	35.28	35.61	35.02	35.51	36.99
18:3	1.63	1.76	1.70	1.77	1.95
20:0	0.52	0.64	0.63	0.66	0.68
20:1	1.05	0.76	0.86	0.56	0.00
22:0	0.43	0.53	0.66	0.60	0.24
24:0	0.48	0.71	0.84	0.85	0.37

Table 3. Tocopherol, tocotrienol, and oryzanol composition of rice bran oil spreads.Concentrations are in $\mu\text{g/g}$.

	Sample 1	Sample 2	Sample 3	Sample 4	Crude RBO
α -tocopherol	186.1	207.03	162.25	189.28	329.90
α -tocotrienol	111.17	126.23	95.46	114.87	229.10
β -tocopherol	7.98	8.92	7.21	8.64	14.40
γ -tocopherol	74.78	88.42	70.31	77.86	116.95
γ -tocotrienol	291.55	340.7	254.5	301.67	511.54
δ -tocopherol	2.20	2.89	2.59	2.12	3.96
δ -tocotrienol	9.33	12.62	8.43	10.34	15.13
γ -oryzanol	2646	3027	2967	3071	13017

Table 4. Phytosterol composition of rice bran oil spreads. Concentrations are in $\mu\text{g/g}$.

	Sample 1	Sample 2	Sample 3	Sample 4	Crude RBO
Squalene	18972.44	16559.27	20320.65	16624.70	1574.40
Campesterol	3956.62	4264.43	4195.91	4241.02	2481.64
Campestanol	430.34	461.95	402.62	444.35	247.10
Stigmasterol	3538.16	3716.27	3905.70	3783.36	1834.06
Sitosterol	6894.91	8828.64	7594.81	8414.78	6314.98
Sitostanol	532.25	607.23	618.31	583.62	350.00
Δ -5-Avenasterol	841.82	897.73	882.98	936.57	616.18
Cycloartenol	6559.05	7318.47	6752.17	7217.47	4772.95
Δ -7-Avenasterol	794.31	684.77	929.05	968.34	301.21
24-methylenecycloartanol	8012.03	8910.93	8330.70	8698.27	5570.29

Table 5. Physical properties of rice bran oil spreads.

Property	Sample 1	Sample 2	Sample 3	Sample 4	Crude RBO
SFC of cold slurry [%]	0.87	0.79	1.24	0.92	-
SFC of final product [%]	29.10	27.89	28.41	27.64	1.96
Molar proportion: wax	0.37	0.34	0.37	0.33	-
Molar proportion: TAG	0.63	0.66	0.63	0.67	-
Dropping point [°C]	66.37	66.20	64.90	63.02	-
T1 [onset, °C]	-26.77	-27.33	-26.87	-27.67	-
T1 [end, °C]	6.98	6.92	7.41	7.29	-
T2 [onset, °C]	70.65	70.13	70.83	70.11	-
Heat of melting at 75-79°C [J/g]	57.55	50.62	56.61	47.55	-
G' [Pa]	$5.16 \cdot 10^5$	$4.76 \cdot 10^5$	$8.89 \cdot 10^5$	$3.97 \cdot 10^5$	-
G'' [Pa]	$8.95 \cdot 10^4$	$9.02 \cdot 10^4$	$1.72 \cdot 10^5$	$7.93 \cdot 10^4$	-

Table 6. Yields of spreadable rice bran products.

Sample	Incubation Temperature [°C]	Time	Yield [%]
1	4	30	5.27
2	4	60	5.84
3	-20	30	5.80
4	-20	60	6.03

Example 2

[0060] This example demonstrates the use of this spread through two avenues: as an oil additive during frying and also as a shortening-like ingredient. First, as an oil additive, the rice bran oil-derived spread was added to refined soybean oil at concentrations ranging from 0.25 - 2% by weight, and its ability to delay lipid oxidation was monitored. These oil formulations were then evaluated by a sensory panel through tortilla chips fried in the enhanced oils. Second, the rice bran oil-derived ingredient was evaluated by a sensory panel as a fat replacement in a bread recipe and in granola. In both cases, acceptable levels of the rice bran oil ingredient were established. Thus, it is possible to incorporate this phytosterol-enriched structured lipid into everyday products.

Materials and Methods

Materials

[0061] Hexane-extracted, alkali-refined, bleached and deodorized soybean oil (SBO) was obtained from a commercial processor. Crude rice bran oil (REO) was obtained from Riceland Foods (Stuttgart, AR). Oils were stored at -20°C prior to use.

Solvent fractionation to produce spreadable products from rice bran oil

[0062] Rice bran oil was brought to room temperature prior to fractionation. Acetone was chilled in a standard freezer at -20°C for several hours prior to fractionation in a stoppered Erlenmeyer flask. Rice bran oil (80 g per 200 mL acetone) was added to a beaker, and cold acetone was slowly added. The mixture was stirred briefly on a stirplate before being held at -20°C for 60 minutes. The cold slurry was then filtered through Whatman No. 1 filter paper using a Buchner funnel. The filter paper was scraped periodically to harvest the product, which was dried under vacuum for a minimum of 48 hours. For products prepared for sensory panels, only food grade lab equipment was used.

Unsaponifiable Matter Analysis

[0063] Determination of unsaponifiable matter was conducted according to AOCS method Ca 6b-53 (AOCS, *ibid*). Samples were analyzed in triplicate, and sample blanks were run in duplicate.

Heating Studies

[0064] Flat-bottomed 10 mm glass NMR tubes were filled to 4 cm $\pm$ 1 cm with oil samples. Tubes were then randomly positioned

in a heating block in a forced-air oven maintained at $180 \pm 1^\circ\text{C}$ for 0, 2, 4, 6, 8, 16, or 24 hours. Three tubes were heated for each time point. After the specified amount of time, the tubes were removed from the oven, cooled briefly at room temperature, and the headspace was filled with argon and tubes were capped until further measurements could be made.

Total Polar Compounds

[0065] Total polar compounds were determined according to a micro method developed by Dobarganes et al. and modified in our laboratory. This method is a modification of the AOCS official method Cd 20-91(97) (AOCS, *ibid*). Oils were separated on a 1 g silica SepPak (Waters Corporation). The column was conditioned with 10 mL of 90:10 (v/v) petroleum ether:diethyl ether, and then 1 mL of a 25 mg/mL oil solution in CHCl_3 was loaded onto the SepPak. The nonpolar fraction was eluted with an additional 10 mL of the 90:10 petroleum ether to diethyl ether mixture, followed by 10 mL of 50:50 (v/v) chloroform:methanol to elute the polar fraction. Fractions were dried under nitrogen and gentle heat for several hours and weighed upon dryness. Three samples were analyzed for each time point, and each sample was analyzed in duplicate.

Polymer Determination

[0066] Total triacylglycerol dimers, trimers, and higher-molecular weight polymers were analyzed by high performance size exclusion chromatography using a Shimadzu LC20AT pump equipped with membrane degasser and autosampler. 10 μL of a 5.0 mg/mL solution of each oil in methylene chloride was injected onto three successive PLgel Mixed E columns (3 mm, 100 Å pore size, 30067.5 mm; Polymer Labs, Amherst, MA, USA). The mobile phase used was methylene chloride at a flow rate of 0.8 mL/min. Peaks

were detected with an evaporative light scattering detector (Sedex55; S.E.D.E.R.E., France) operated at a temperature of 40°C with the nebulizer gas (ultra-pure N₂) pressure set to 2.5 bar and the gain set at 4. Instrument control and data analysis were performed by Shimadzu EZStart Chromatography Software Version 7.3. Three samples were analyzed for each time point, and each sample was analyzed in duplicate.

Oil Stability Index

[0067] The OSI at 110°C was determined according to AOCS Official Method Cd 12b-92 (AOCS, *ibid*). A Metrohm (Herisau, Switzerland) 743 Rancimat was used, which calculated OSI values based on induction time. All samples were measured in triplicate.

Sensory Panels

Evaluation of rice bran oil spread as an oil additive

Preparation of panel and samples for evaluation of frying oils

[0068] A 17 member analytical panel, trained and experienced in evaluating frying oils was used for discrimination tests of chips fried in soybean oil and chips fried in soybean oil with rice bran spread added.

[0069] Samples were prepared as follows: Corn tortillas were obtained from a local grocery store and cut into eighths. Batches of 40 g (20-22 chips) were fried in either soybean oil or soybean oil to which rice bran spread had been added (0.25-2% by weight). The total weight of the frying oil was 900 g. Oils were heated to 180°C using a Fry Daddy electric deep fryer (National Presto Industries, Inc.) and maintained at that temperature with a J-KEM temperature controller. Chips were

submerged in the hot oil for 2 minutes, at which point the basket was removed from the fryer and drained. Chips were cooled on paper towels. Cooled chips were pooled and ground with a mortar and pestle before being placed into beakers for panelist evaluation.

Sensory evaluation of rice bran oil spreads in frying oils

[0070] Each concentration of rice bran spread in soybean oil was evaluated twice, on separate occasions, for a total of ten triangle test sessions. Testing was performed in individual sensory testing booths equipped with red lights to mask any color differences between samples. Triangle tests were administered as described in Meilgaard et al. (*Sensory Evaluation Techniques*. CRC Press, Boca Raton, FL 1999). A random number generator (Research Randomizer) was used to generate three digit codes for sample identification. Sample combinations (AAB, ABA, BAA, etc.) were also randomized among panelists.

Evaluation of rice bran oil spread in white bread

Preparation of panel and samples for evaluation of white bread

[0071] A 20-22 member panel trained in discrimination testing was used to evaluate bread. Two different experimental breads were prepared: one in which there was 50% fat replacement and one at 25% fat replacement. Each experimental bread was evaluated in reference to a control bread, which contained no rice bran oil ingredients.

Breads were prepared as follows:

Control Recipe	25% Fat Replacement	50% Fat Replacement
240 mL milk	240 mL milk	240 mL milk
1 egg	1 egg	1 egg
28 g margarine	21 g margarine	14 g margarine
	7 g rice bran oil spread	14 g rice bran oil spread
67 g granulated sugar	67 g granulated sugar	67 g granulated sugar
3 g salt	3 g salt	3 g salt
375 g all purpose flour	375 g all purpose flour	375 g all purpose flour
4 g active dry yeast	4 g active dry yeast	4 g active dry yeast

Ingredients were added to a standard one-load bread machine in the order listed. Loaves were baked using the sweet bread setting with the crust set to medium. After baking, the loaf was briefly cooled in the bread machine and then removed and placed on a cooling rack. After further cooling, breads were wrapped until sensory testing.

Sensory evaluation of rice bran oil spread in bread

[0072] Testing was performed in individual sensory testing booths equipped with red lights to mask any color differences between samples. Triangle tests were administered as described in Meilgaard et al. (*ibid*). A random number generator (Research Randomizer) was used to generate three digit codes for sample identification. Sample combinations (AAB, ABA, EAA, etc.) were also randomized among panelists.

Evaluation of rice bran oil spread in granola

Preparation of panel and samples for evaluation of granola

[0073] A 19–20 member panel trained in discrimination testing was used to evaluate granola. Two different experimental granolas were prepared: one in which there was 17% fat replacement and one at 25% fat replacement. Each experimental granola was evaluated in reference to a control granola, which contained no rice bran oil ingredients.

Granolas were prepared as follows:

Control Recipe	17% Fat Replacement	25% Fat Replacement
206 g oats	206 g oats	206 g oats
70 g dark brown sugar	70 g dark brown sugar	70 g dark brown sugar
50 g peach preserves	50 g peach preserves	50 g peach preserves
80 g butter	67 g butter	60 g butter
	13 g rice bran spread	20 g rice bran spread
1 tsp vanilla (~3 g)	1 tsp vanilla (~3 g)	1 tsp vanilla (~3 g)
½ Tbsp cinnamon (~ 4 g)	½ Tbsp cinnamon (~ 4 g)	½ Tbsp cinnamon (~ 4 g)

Preserves, brown sugar, butter, and rice bran spread (if applicable) were melted together in a large pan on the stove at 160–165°C. The pan was then removed from heat, and vanilla and cinnamon were added. Oats were then added and the mixture was stirred until the oats were uniformly coated. The mixture was baked at 180°C for 35 minutes, stirring every 3–5 minutes to prevent uneven browning. Granola was cooled at ambient temperature for 10–15 minutes and then placed in airtight containers.

Sensory evaluation of rice bran oil spread in granolas

[0074] Testing was performed in individual sensory testing booths equipped with red lights to mask any color differences between samples. Triangle tests were administered as described in Meilgaard et. al (*ibid*). A random number generator (Research Randomizer, <http://www.randomizer.org/>) was used to generate three digit codes for sample identification. Sample combinations (AAB, ABA, BAA, etc.) were also randomized among panelists.

Statistical Analyses for sensory evaluations

[0075] Correct responses were tabulated and compared to calculated threshold values for correct responses corresponding to $P = 0.05$ (Meilgaard et al., *ibid*).

Results and Discussion

Composition of rice bran oil spreads from rice bran oil

[0076] An overall chemical characterization of the rice bran oil spread described here has been published previously and included fatty acid composition, free fatty acids, tocopherol and tocotrienol analysis, phytosterol analysis, and wax concentration. Additional chemical characterization is presented here. Because it has been suggested that the beneficial effects of rice bran oil products can be linked to the content of unsaponifiable matter in rice bran oil (Lee et al.: Beneficial effect of the unsaponifiable matter from rice bran on oxidative stress in vitro compared with α -tocopherol, J. Sci. Food Agric. 2005, 85:493-498; Eady et al.: Consumption of a plant sterol-based spread derived from rice bran oil is effective at reducing plasma lipid levels in mildly hypercholesterolaemic individuals, Br J Nutr. 2011, 1-12), unsaponifiable matter was determined in both the crude rice bran

oil and resulting rice bran oil spreads. It was found that unsaponifiable matter constitutes approximately 3.66% of crude rice bran oil. The unsaponifiable content of crude rice bran oil is in agreement with previously reported values of 3-5% (Rana et al.: In vivo antioxidant potential of rice bran oil (RBO) in albino rats, Indian Journal of Physiology and Pharmacology. 2004, 48, 428-436).

Oxidative Stability of oils with rice bran oil spread

[0077] Because crude rice bran oil contains many compounds with antioxidant activity, it was hypothesized that the spreads derived from rice bran oil would also have antioxidant activity when combined with traditional frying oils. Rice bran oil spreads were added to refined, bleached, deodorized soybean oil at concentrations ranging from 0.25% to 2%. Soybean oil was selected as the oil matrix because it is relatively inexpensive, readily available, and commonly used for frying. The oils were then heated to 180°C, frying temperature, in an oven, and lipid oxidation was monitored via total polar compounds and polymer analysis. While prolonged heating of edible oils at 180°C cannot replicate all of the complex processes that take place during frying, heating of oil to frying temperatures has been shown to be both reproducible and sensitive for determining quality differences in edible oils (Warner & Kwolek: Effects of antioxidants, methyl silicone and hydrogenation on room odor of soybean cooking oils, Journal of the American Oil Chemists' Society. 1985, 62:1483-1486; Sims & Kanuk: Sterol additives as polymerization inhibitors for frying oils, Journal of the American Oil Chemists' Society. 1972, 49:298-301; Gordon: The effect of sterols on the oxidation of edible oils, Food Chemistry. 1983, 10:141-147; and Evans et al.: Room odor

evaluation of oils and cooking fats, *Journal of the American Oil Chemists' Society*. 1972, 49:578-582). The degree of polymerization during heating of oils is generally quantified by total polar compounds and/or high performance size exclusion chromatography (Gertz: Chemical and physical parameters as quality indicators of used frying fats, *Eur. J. Lipid Sci. Technol.* 2000, 102:566-572).

[0078] Antioxidative effects provided by the rice bran oil spreads were not detected by total polar compounds analysis. Mixtures of soybean oil with added rice bran oil spread oxidized at a similar rate to soybean oil alone (Figure 6). Absolute concentrations of polar compounds were also not useful, as the presence of rice bran oil spread increased the measured total polar compounds, even in the absence of heating. The degree of polymerization was also determined via high performance size exclusion chromatography (Figure 7). TAGs decreased and dimer concentration increased in all oil mixtures with increasing heating time, again at similar rates to soybean oil alone. However, the 0.25% rice bran oil spread mixture consistently showed higher TAG concentrations and lower dimer concentrations than the control and other mixtures, indicating that rice bran oil spread may have a protective effect on triacylglycerols at 0.25%.

[0079] Oxidative stability (OSI) of the oils was also assessed via Rancimat. Here antioxidant activity by rice bran oil spread under Rancimat conditions was observed in a dose-dependent fashion, with the 2% mixture having the highest oxidative stability index at 13.2 hours (Figure 8). The temperature at which OSI was assessed was 110.9°C, considerably lower than the 180°C used to carry out the heating studies. This

may explain the contrast between the weak antioxidant activity suggested by total polar compounds and TAG concentrations and the dose-dependent antioxidant activity suggested by OSI.

Sensory Analyses

[0080] The antioxidant and textural properties of the rice bran oil spread suggested two potential avenues for incorporation into foods as a functional ingredient: first, OSI data suggest that rice bran oil spreads could be added to frying oils to extend the fry life of the oil. Second, the texture of the spread is similar to a shortening, implying that the spread might be suitable as a substitute for shortenings or other fats in baked goods. Jennings and Akoh showed that consumers would be willing to purchase products incorporating a rice bran oil structured lipid (Jennings et al.: Food Applications of a Rice Bran Oil Structured Lipid in Fried Sweet Potato Chips and an Energy Bar, J. Food Qual. 2010, 33:679-692). A total of 3 investigations were performed using a sensory panel: rice bran oil spread as an oil additive in soybean oil during frying, and as a fat replacer in two baked goods, white bread and granola.

[0081] In the first series of studies, the sensory properties of frying oils using rice bran oil spread as an additive were investigated. A total of 5 concentrations were tested, each on two separate occasions, for a total of 10 discrimination tests utilizing fried tortilla chips. In discrimination testing, the number of correct responses required to establish a statistically significant difference between samples depends on the number of independent assessors. Over the course of the 10 sessions, the number of panelists varied from 15 to 17, which corresponded to a threshold value of panelists scoring correctly of 9 (n=15 and n=16) or 10 (n=17). At concentrations of 1.5%

and 2% rice bran oil spread by weight, the panel as a whole was able to distinguish tortilla chips fried in these oils from chips fried in a control SBO containing no rice bran oil spread (Figure 9). At intermediate concentrations (1.0 and 0.5%), the threshold value was reached only half of the time. At 0.25% rice bran oil spread, the sensory panel was unable to reliably distinguish between samples. OSI results suggest at even at 0.25%, there is some benefit afforded by the rice bran oil spread.

[0082] The use of the rice bran oil spread as a functional ingredient in baked goods was also explored. The spread was incorporated as a butter replacement in a white bread at 25% and 50% and in a baked granola at 17% (one-sixth) and 25% replacement. Sensory panels were conducted on one occasion for each concentration. For white bread, the 25% and 50% replacement trials had 20 and 22 participants, respectively. At 25% rice bran oil spread, the panel was unable to distinguish between the experimental sample and a control bread (7 correct answers out of 20). However, at 50% rice bran oil spread, 14 correct responses out of 22 total responses were given, exceeding the threshold value of 12 correct responses (Figure 10). Therefore the sensory threshold of this rice bran oil spread in a white bread lies between 25% and 50% butter replacement. For the granola studies, the 17% and 25% substitution trials had 20 and 19 participants, respectively. At 25% replacement, the panel exactly met the threshold value of 11 correct responses required to establish a significant difference between samples. At 17% (one-sixth) replacement, the sensory panel was unable to distinguish between the control and experimental granola samples: 11 correct answers were required and only 5 correct answers were given (Figure 10). Therefore,

the sensory threshold of rice bran oil spread in granola is between 17% and 25% fat replacement.

[0083] Acceptability ratings were determined for the discrimination tests involving the white bread and baked granola (Figure 11). Panelists were asked to rate each of the three samples in the triangle test as acceptable or unacceptable. In all cases, a majority of the panelists indicated that the experimental sample(s) as acceptable, although at the proportion of butter replacement increased, the acceptability ratings decreased. At 17% butter replacement in granola, 100% of the panelists determined the experimental sample(s) to be acceptable. The high acceptability ratings from all four scenarios indicate that rice bran oil derived spreads can be incorporated as functional ingredients into foods while still maintaining high product acceptability.

Conclusions

[0084] In conclusion, we have evaluated a rice bran oil derived spread for its suitability as a functional ingredient in a frying oil and in two types of baked goods. In each case, a sensory threshold for this ingredient was established. Acceptability ratings for rice bran oil spreads in baked products were consistently above 85% and in one case, 100% of panelists rated the experimental sample as acceptable. Rice bran oil spread was found to impart antioxidative activity, established by total polar compounds, polymers, and OSI, at all of the sensory thresholds determined. Together the data suggest that this material would be suitable for further development as a functional food ingredient.

[0085] It is understood that the foregoing detailed description is given merely by way of illustration and that modifications and variations may be made therein without departing from the spirit and scope of the invention.

I claim:

1. A method for making a spreadable rice bran oil product comprising:

- a) providing a rice bran oil;
- b) contacting said rice bran oil with acetone to form a first oil/acetone mixture under conditions to establish a temperature of the mixture between -15 to -30°C, said acetone being provided in said mixture in an amount effective to extract wax, some triacylglycerols, and phytosterols from the oil as a solid phase precipitate;
- c) recovering said solid phase precipitate from said mixture.

2. The method of claim 1 further comprising maintaining the temperature of said mixture between -15 to -30°C for a period of time until said solid phase precipitate comprises a wax to triacylglycerol molar ratio between 1:4 and 2:3.

3. The method of claim 2 wherein solid phase precipitate comprises a wax to triacylglycerol molar ratio between 1:3 and 2:3.

4. The method of claim 2 wherein said temperature of said mixture is between -15 to -28°C.

5. The method of claim 2 wherein said temperature of said mixture is between -20 to -25°C.

6. The method of claim 1 wherein said amount of said acetone is greater than or equal to 1 ml of acetone per gram of said oil.

7. The method of claim 6 wherein said amount of said acetone is greater than or equal to 1.5 ml of acetone per gram of said oil.

8. The method of claim 6 wherein said amount of said acetone is greater than or equal to 2 ml of acetone per gram of said oil.

9. The method of claim 6 wherein said amount of said acetone is greater than or equal to 2.5 ml of acetone per gram of said oil.

10. The method of claim 1 wherein said rice bran oil has not been refined, dewaxed or hydrogenated.

11. The method of claim 1 wherein said rice bran oil comprises crude rice bran oil.

12. The method of claim 1 wherein said contacting is in the absence of additional solvent.

13. The method of claim 1 further comprising drying said solid phase precipitate recovered from said mixture.

14. The method of claim 1 wherein said triglycerides (preferentially extracted to provide a higher relative amount in the solid phase precipitate than the rice bran oil) extracted from said rice bran oil into said solid phase comprise one or more of myristic acid (14:0), palmitic acid (16:0), behenic acid (22:0) and lignoceric acid (24:0).

15. The method of claim 1 wherein said recovering is selected from the group consisting of filtration, settling, decantation and centrifugation.

16. A spreadable rice bran oil product comprising the solid phase precipitate produced by claim 1.

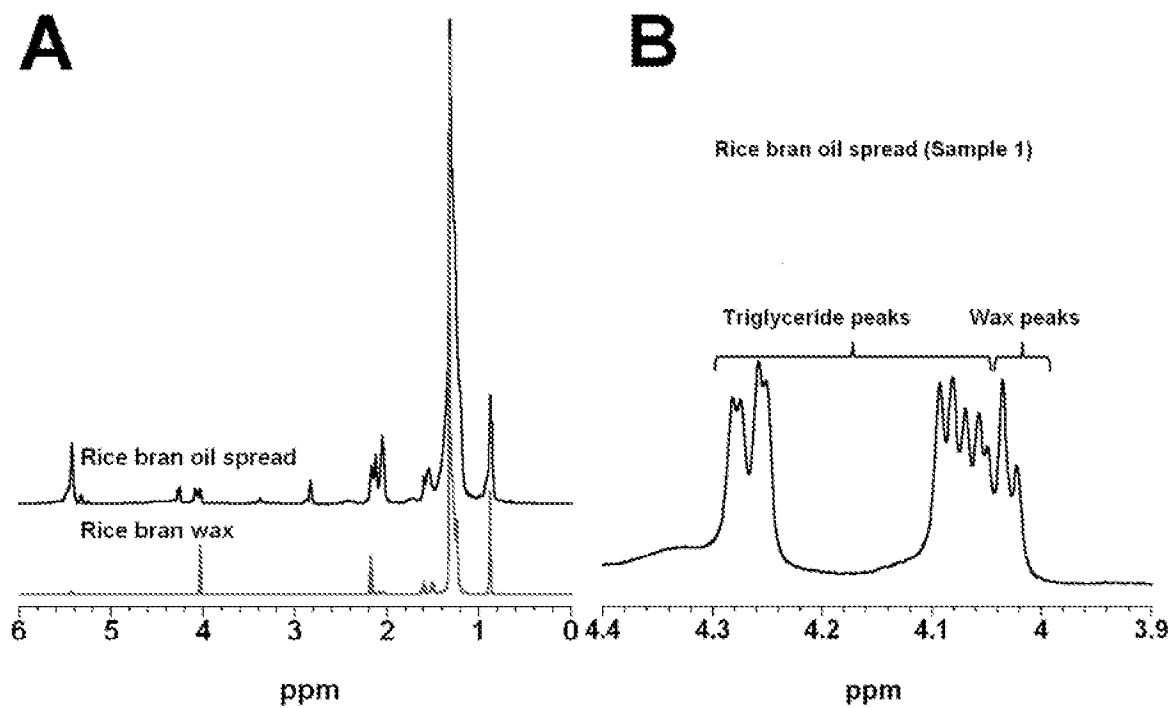
17. A spreadable rice bran oil product comprising the solid phase precipitate produced by claim 2.

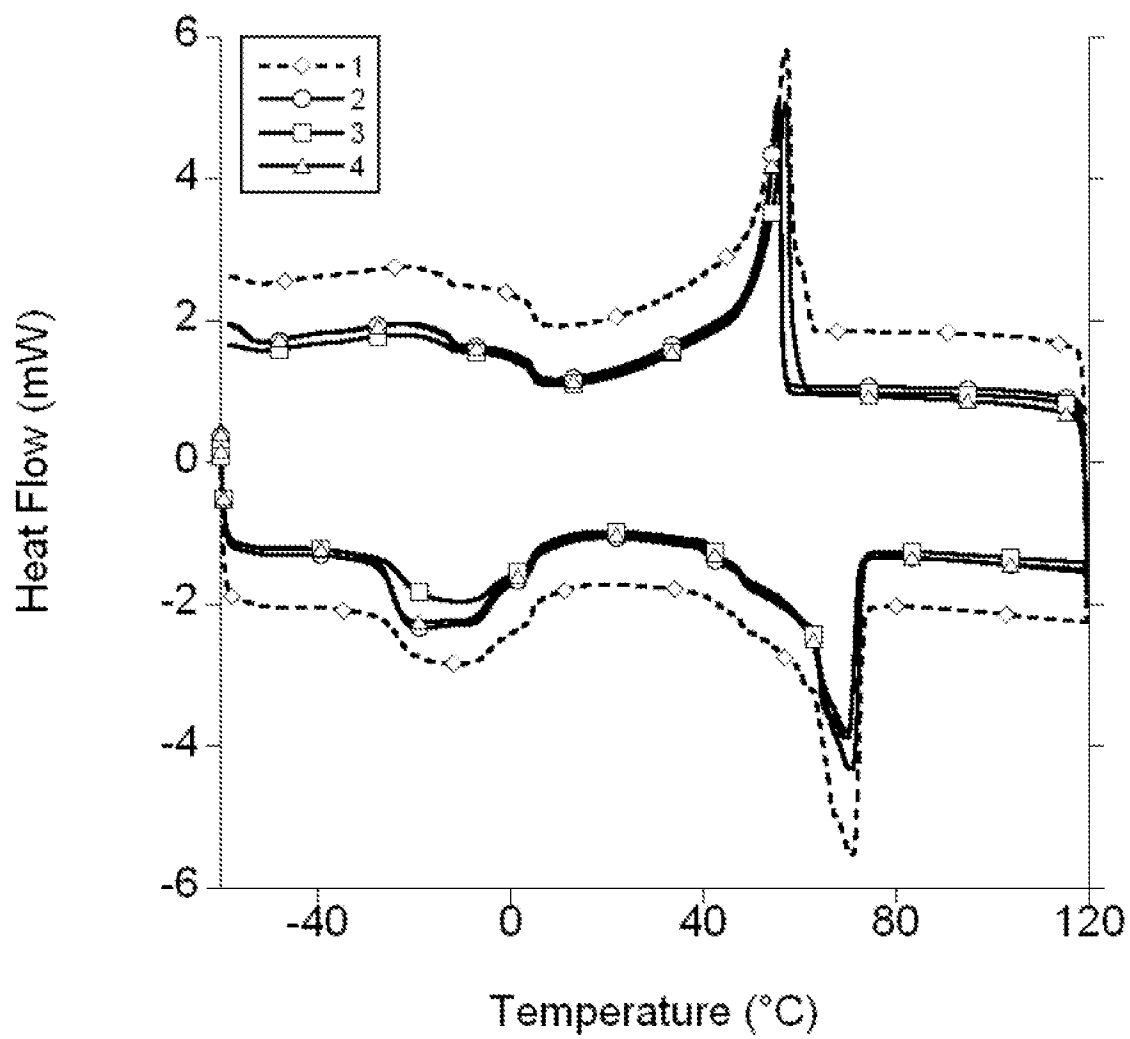
18. A spreadable rice bran oil product comprising the solid phase precipitate produced by claim 3.

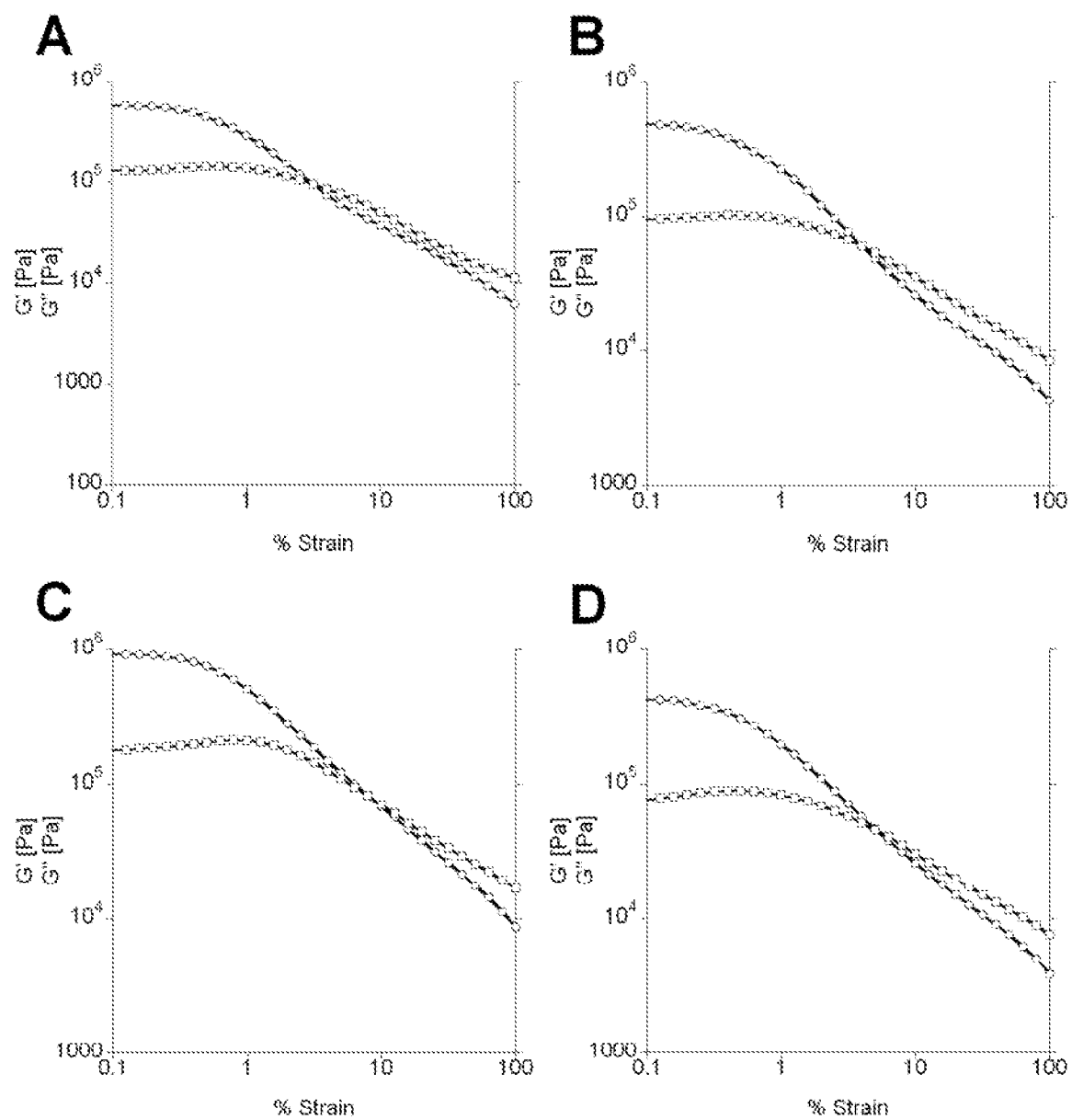
19. A spreadable rice bran oil product comprising the solid phase precipitate produced by claim 5.

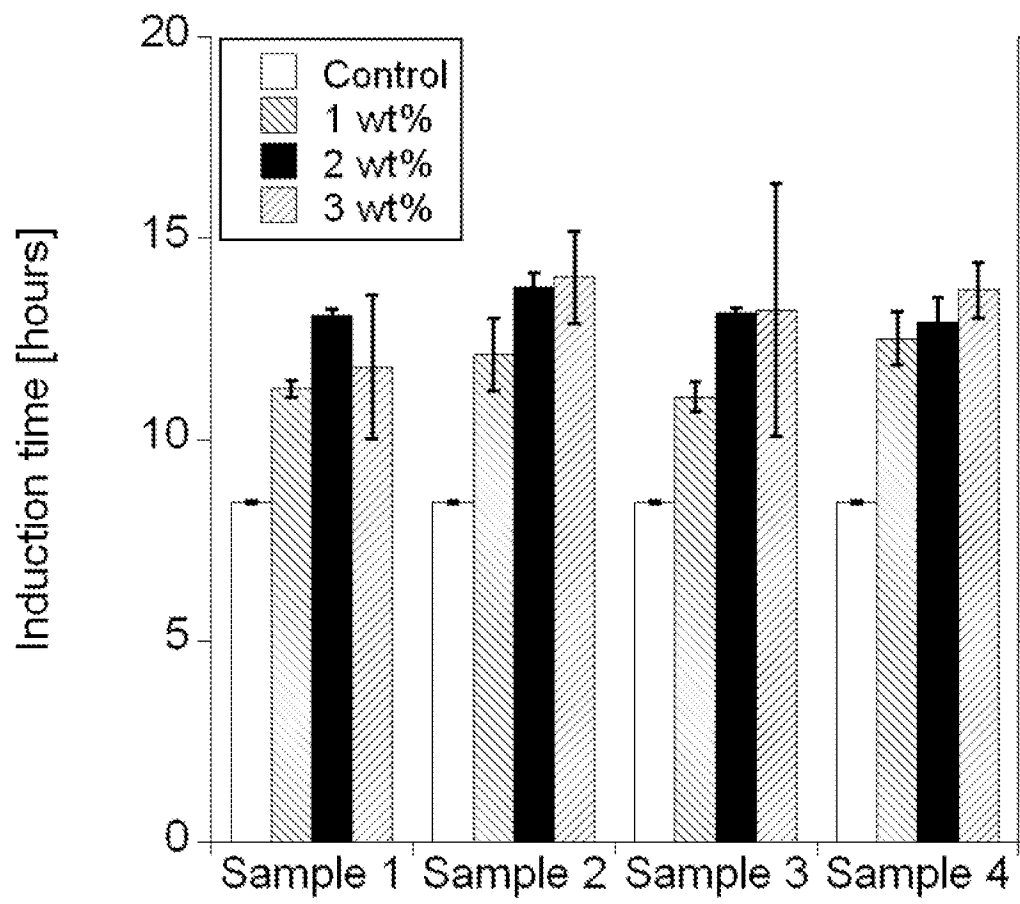
20. A spreadable rice bran oil product comprising the solid phase precipitate produced by claim 10.

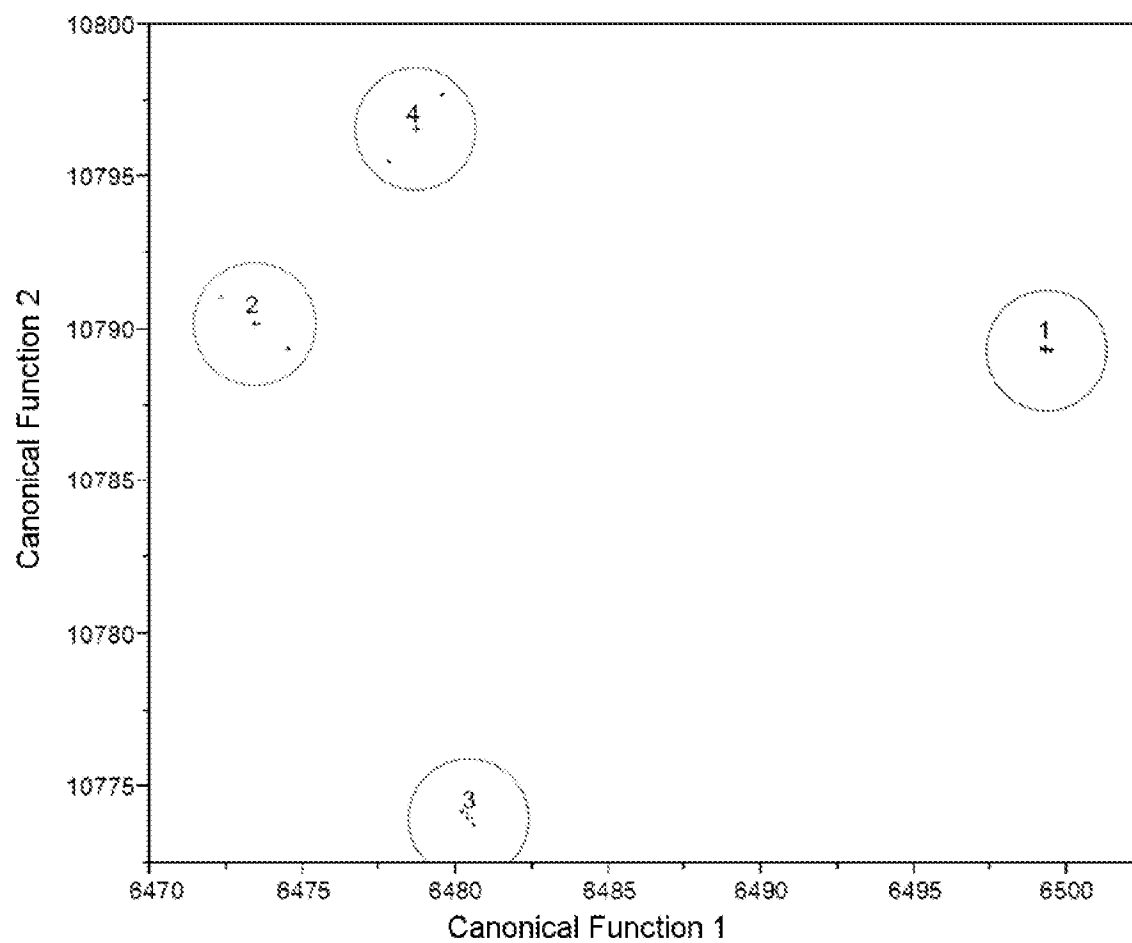
21. A spreadable rice bran oil product comprising the solid phase precipitate produced by claim 11.

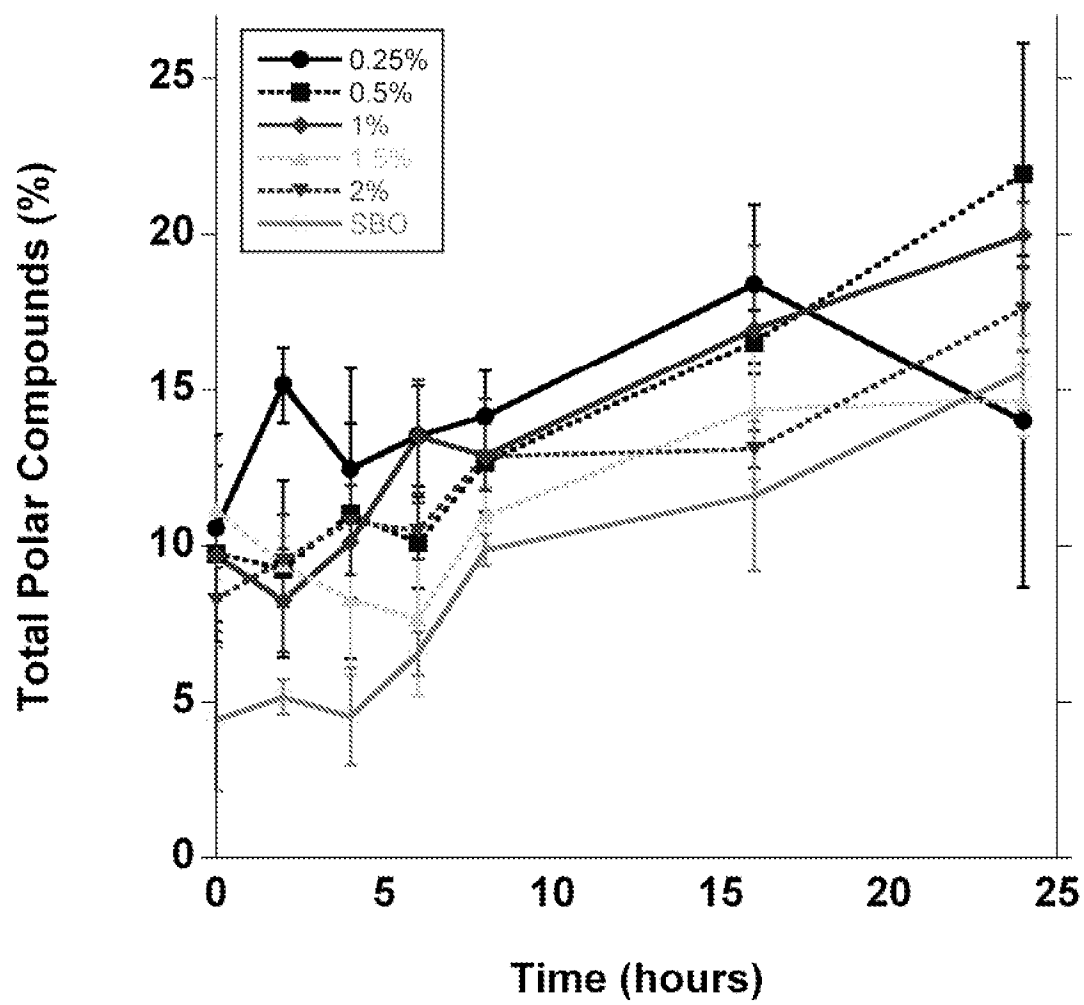
**Figure 1**

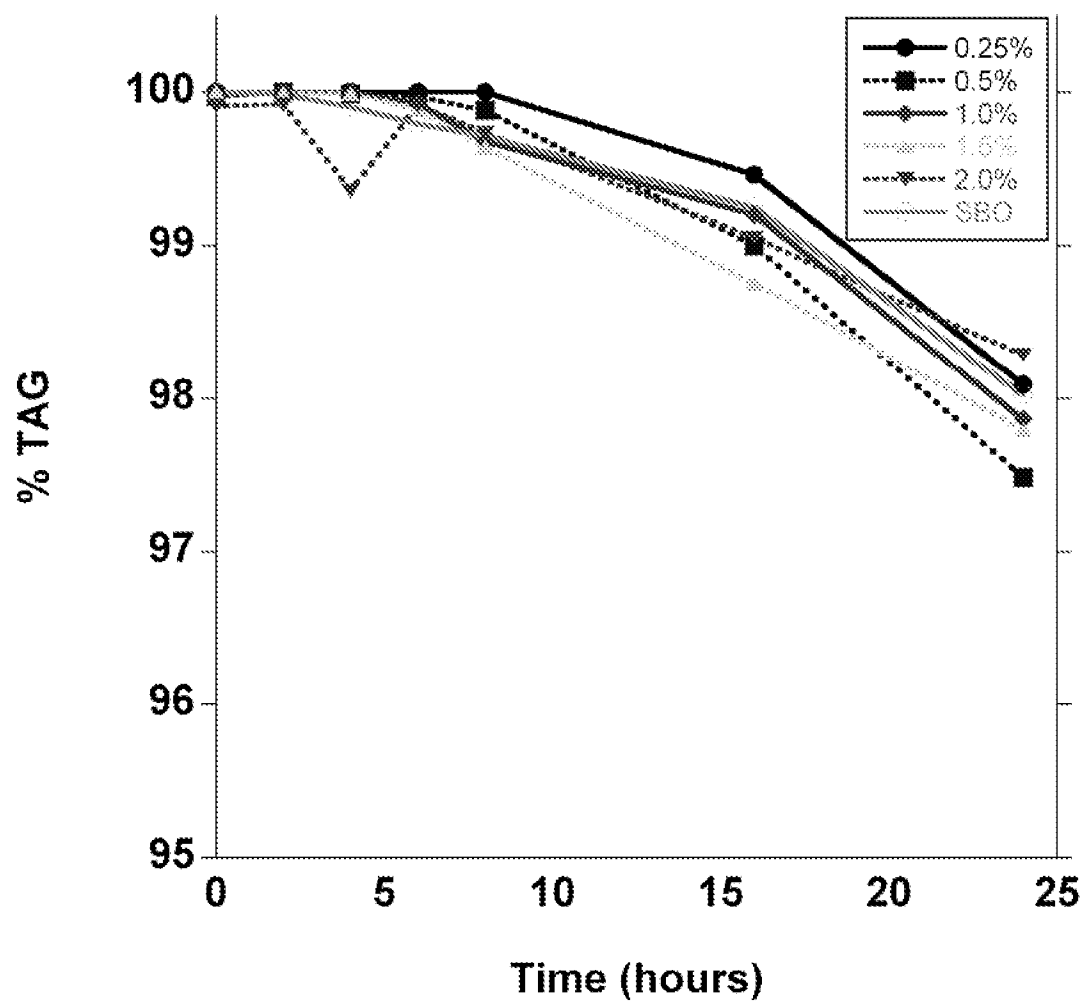
**Figure 2**

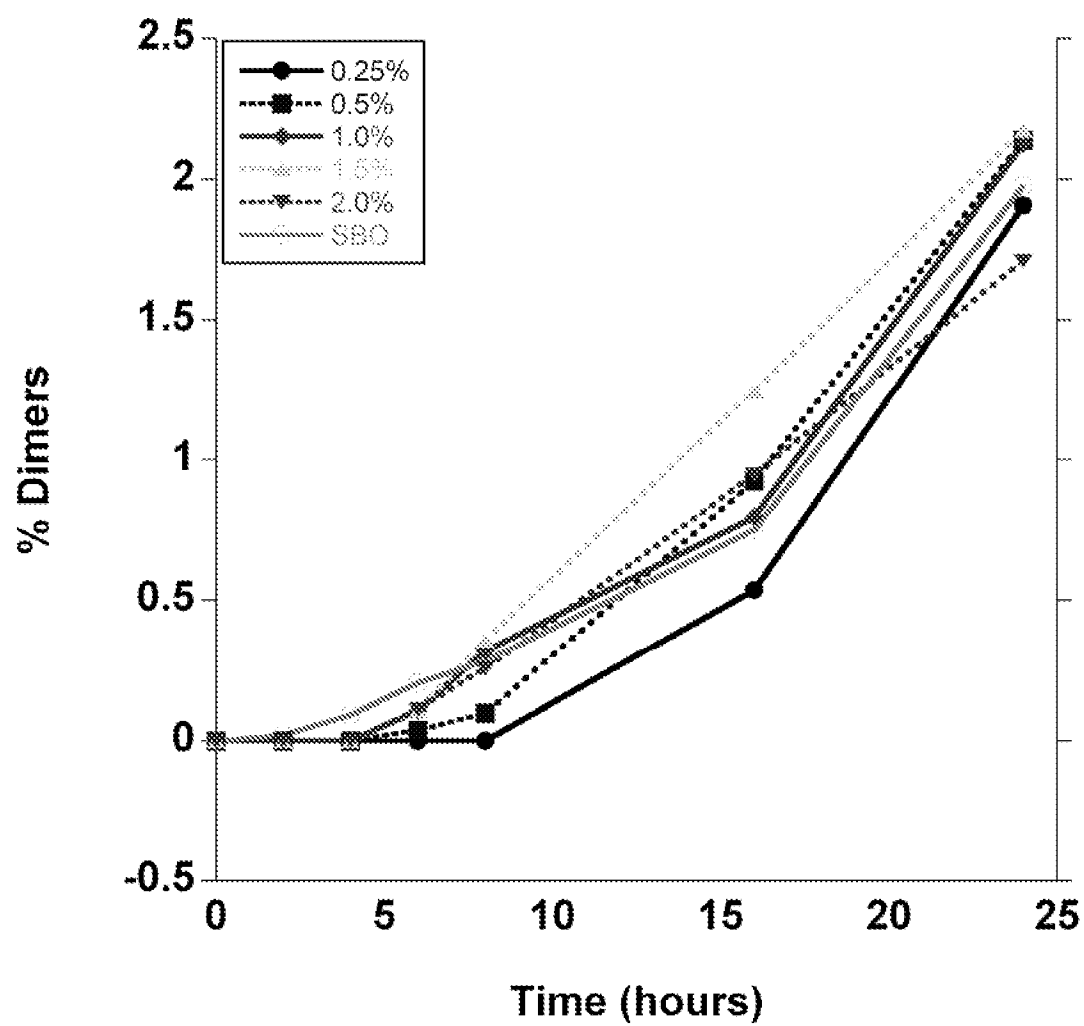
**Figure 3**

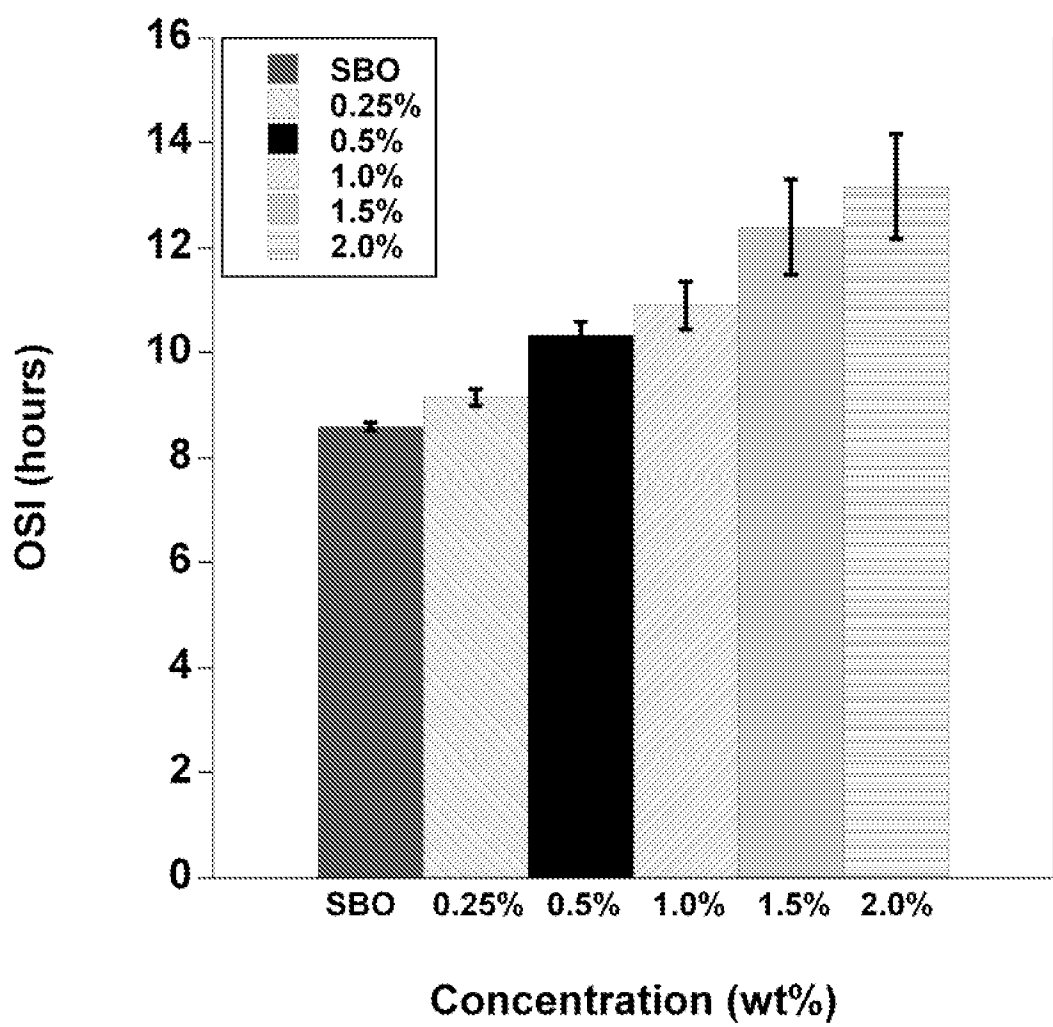
**Figure 4**

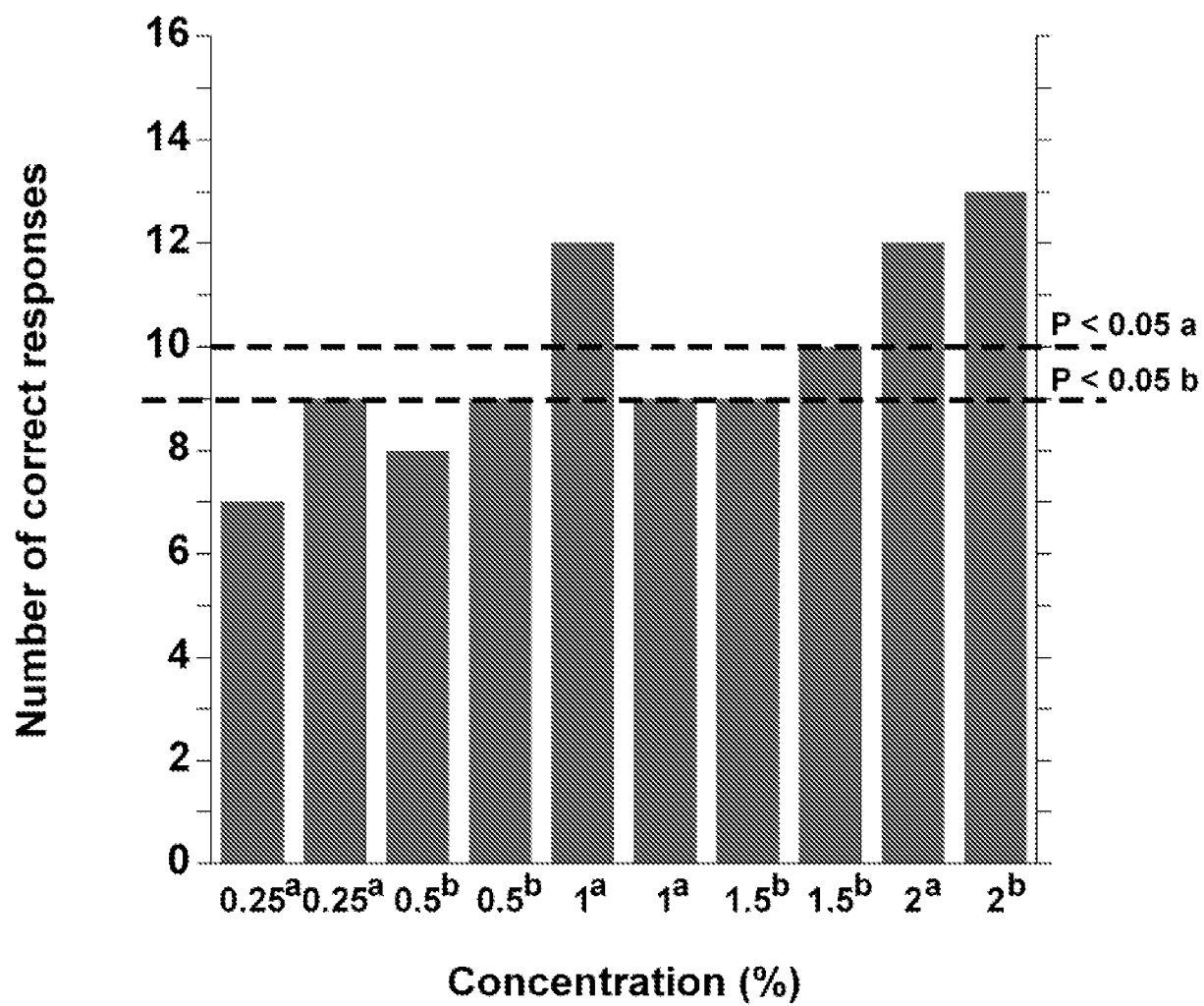
**Figure 5**

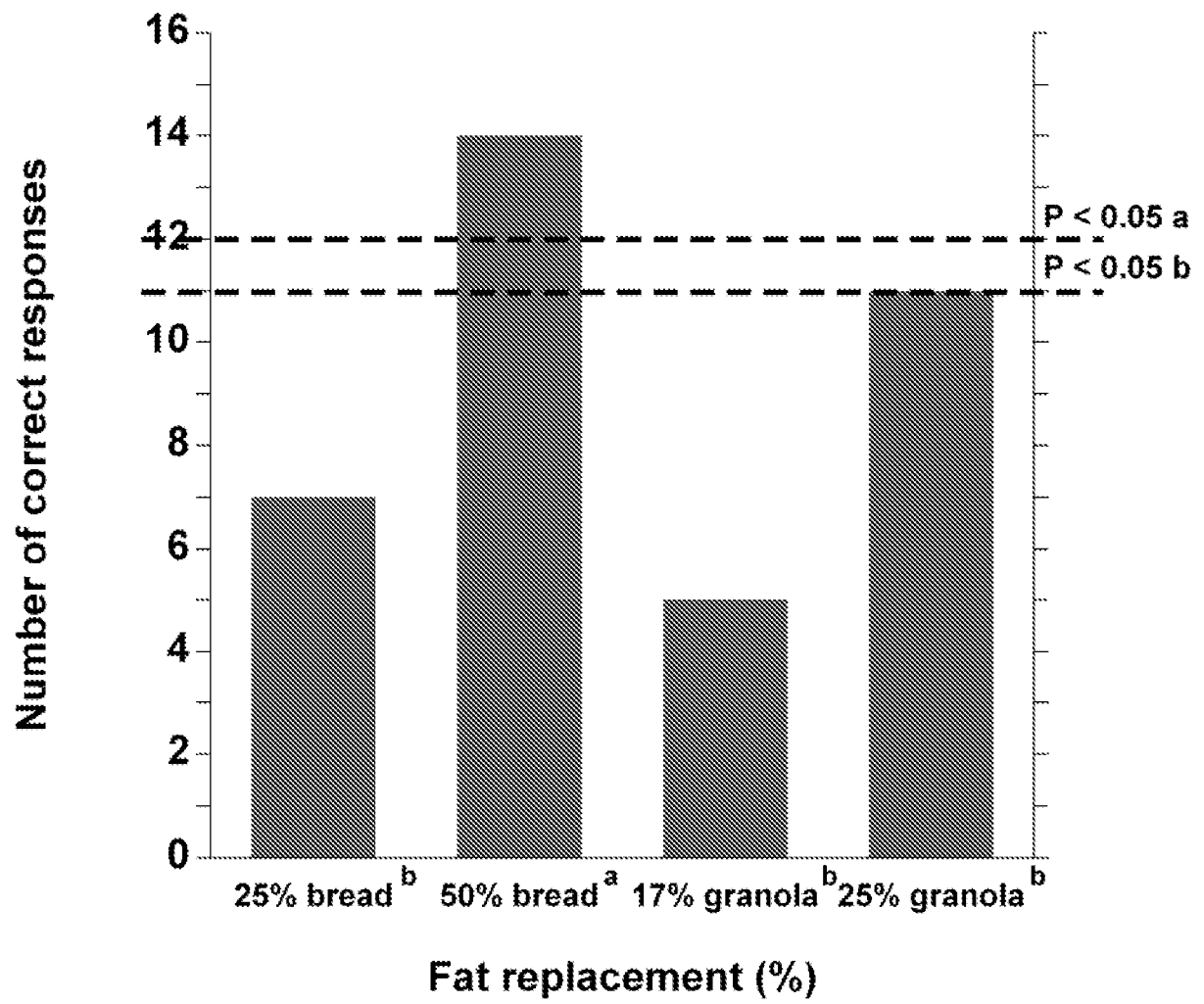
**Figure 6**

**Figure 7a**

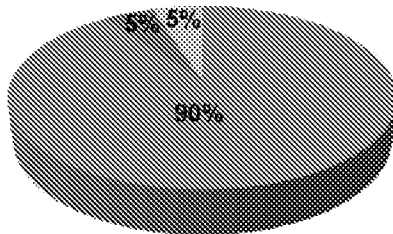
**Figure 7b**

**Figure 8**

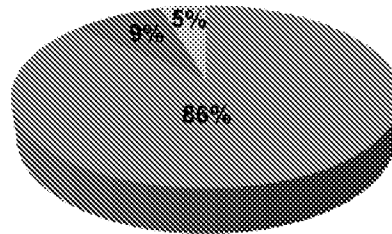
**Figure 9**

**Figure 10**

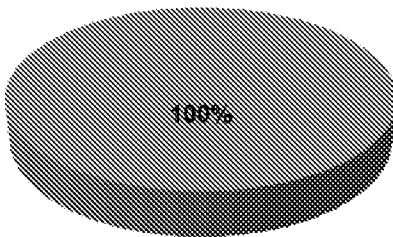
Bread with 25% Butter Replacement



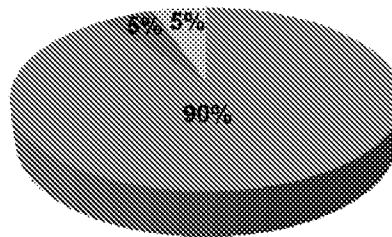
Bread with 50% Butter Replacement



Granola with 17% Butter Replacement



Granola with 25% Butter Replacement



■ Acceptable
■ Unacceptable
■ Neither/No Response

Figure 11

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2014/043385**A. CLASSIFICATION OF SUBJECT MATTER****C08L 91/06(2006.01)i, C11B 1/10(2006.01)i**

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C08L 91/06; C11B 1/00; U11B 700; A61Q 5/00; C07F 9/02; A61K 36/00; A61K 35/78; A23D 5/00; C11B 1/10

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Korean utility models and applications for utility models

Japanese utility models and applications for utility models

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

eKOMPASS(KIPO internal) & Keywords: rice, bran, oil, acetone, solid, precipitate, spreadable

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 6552208 B1 (ALANDER et al.) 22 April 2003 See abstract; and claims 1, 2, 4, 5, 14.	1-21
A	US 2008-0138450 A1 (ZIMA et al.) 12 June 2008 See abstract; and claims 1, 2, 13.	1-21
A	US 2004-0192948 A1 (INDIRA et al.) 30 September 2004 See abstract; and claims 1-4.	1-21
A	US 4061798 A (KANEKAE et al.) 6 December 1977 See abstract; and claims 1, 4.	1-21
A	US 8232418 B1 (BILBIE et al.) 31 July 2012 See abstract; and claims 1, 7-9.	1-21



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

23 October 2014 (23.10.2014)

Date of mailing of the international search report

23 October 2014 (23.10.2014)

Name and mailing address of the ISA/KR

International Application Division
Korean Intellectual Property Office
189 Cheongsu-ro, Seo-gu, Daejeon Metropolitan City, 302-701,
Republic of Korea

Facsimile No. +82-42-472-7140

Authorized officer

CHANG, Bong Ho

Telephone No. +82-42-481-3353



INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/US2014/043385

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 6552208 B1	22/04/2003	AT 287436 T	15/02/2005
		AU 1999-46673 A1	20/12/1999
		AU 4667399 A	20/12/1999
		DE 69923309 D1	24/02/2005
		DE 69923309 T2	06/04/2006
		EP 1084215 A1	21/03/2001
		EP 1084215 B1	19/01/2005
		SE 514725 C2	09/04/2001
		SE 9801955 A	03/12/1999
		SE 9801955 D0	02/06/1998
		WO 99-63031 A1	09/12/1999
US 2008-0138450 A1	12/06/2008	AT 402186 T	15/08/2008
		AU 2004-272561 A1	24/03/2005
		CA 2534961 A1	24/03/2005
		CN 1849331 A	18/10/2006
		CN 1849331 C0	13/08/2008
		DE 602004015308 D1	04/09/2008
		DK 1664078 T3	06/10/2008
		EP 1664078 A1	07/06/2006
		EP 1664078 B1	23/07/2008
		ES 2305858 T3	01/11/2008
		JP 2007-505117 A	08/03/2007
		KR 10-2006-0134913 A	28/12/2006
		US 2005-0051419 A1	10/03/2005
		US 2005-0053712 A1	10/03/2005
		US 2009-092686 A1	09/04/2009
		US 7416756 B2	26/08/2008
		US 7491412 B2	17/02/2009
		WO 2005-026189 A1	24/03/2005
US 2004-0192948 A1	30/09/2004	JP 04528493 B2	18/08/2010
		JP 2004-300034 A	28/10/2004
		US 6896911 B2	24/05/2005
US 04061798 A	06/12/1977	None	
US 8232418 B1	31/07/2012	None	