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Original Research Article

Physicochemical, Textural, Thermal Properties of Rice Bran Oil Spread as Alternate Shortening for Bakery Products

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Abstract: The rice bran oil (RBO) can form its semisolid spread through solvent fractionation. The rice bran oil spread (RBOS) is proposed to be included in the production of bakery products. This study compares the physicochemical, textural properties, and thermal properties of RBOS with commercial shortening (CS) and rice bran oil (RBO). Spreadability analysis was conducted using a texture analyzer. There was no significant difference ($p > 0.05$) in firmness and work of shear between CS and RBOS. The fatty acid compositions were determined through GC-MS. The major fatty acids in RBOS were palmitic acid, oleic acid, and linoleic acid, similar to those in RBO but significantly ($p < 0.05$) higher in saturated fatty acid content. RBO contains a high amount of γ-oryzanol and phytosterols, which is beneficial in solid fat structuring. The quantitation of γ-oryzanol was performed using the UV-Vis spectrophotometer while the phytosterol content was analyzed using HPLC. There was no significant difference ($p > 0.05$) in γ-oryzanol between RBO (1,299.88 ± 50.97 mg/ 100 g sample) and RBOS (1,201.86 ± 84.37 mg/ 100 g sample). However, there was a significant difference ($p < 0.05$) in phytosterols between CS (4.17 ± 0.26 mg/ 100 g sample), RBO (247.00 ± 0.89 mg/ 100 g sample) and RBOS (184.16 ± 0.56 mg/ 100 g sample). Besides, thermal analysis of CS and RBOS was carried out using DSC and TGA. The similar textural properties but higher bioactive compounds content make RBOS a suitable choice as alternate shortening for bakery products.

Keywords: Rice bran oil; Spread; Physicochemical; Textural; Thermal

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1. Introduction

Rice bran oil (RBO) is one of the by-products of the extraction of rice bran. RBO is considered one of the superior edible oils compared to other oils because of its ideal fatty acid composition. RBO contains approximately 18–24% saturated fatty acid (SFA), 40–43% monounsaturated fatty acid (MUFA), and 31–35% polyunsaturated fatty acid (PUFA) (Balachandran *et al.*, 2008; Khatoon & Gopalakrishna, 2004). Besides, RBO contains 4.2% unsaponifiable contents (Ghosh, 2007), which are higher than other oils, such as mustard seed oil (0.56–1.01%) (Konukan *et al.*, 2019; Singh, 2018), sunflower oil (0.81%) (Konukan, *et al.*, 2019), peanut oil (0.98–1.52%) (Zahran & Tawfeuk, 2019), rapeseed oil (0.97%) (Konukan, *et al.*, 2019), olive oil (1.50%) (Textron, 2011) and others. The important unsaponifiable matters in RBO include γ-oryzanol, phytosterols, tocopherols, squalene, and fatty alcohols (Ghosh, 2007; Sahu *et al.*, 2018). These unsaponifiable matters in RBO contribute to the hypocholesterolemic properties, reducing low-density lipoprotein and total serum cholesterol in the human body (Lai *et al.*, 2019; Sahu *et al.*, 2018). Besides, antioxidant properties of rice bran were also reported in a few literature (Mingyai *et al.*, 2018; Nagendra Prasad *et al.*, 2011; Wang *et al.*, 2002). Besides protective effects, its smoke point (254°) is high enough to make RBO suitable for cooking and deep frying (Mariod *et al.*, 2014).

Oil fractionation is a processing technique to vary the oil and fat properties, other than interesterification and hydrogenation. The fractionation process has two stages. Firstly, oils and fat are partially crystallized, undergoing gradual cooling to the specific temperature. Then, the solid (stearin) and liquid (olein) fractions were filtered or centrifuged (Kellens *et al.*, 2007). Oil is being fractionated to become few fractions of solid or semisolid state with various melting points and solid fat content. Based on the procedures and separation methods, there are three types of fractionation in the industry: detergent fractionation, dry fractionation, and solvent fractionation (Hasibuan *et al.*, 2018). The detergent fractionation is a cooling crystallization by batch or continuous with the aid of surfactants and fractions separation (Hasibuan *et al.*, 2018). Dry fractionation is a thermomechanical separation process, while solvent fractionation is partial crystallization due to the partial solubility into the specific solvent (Jääskeläinen *et al.*, 2017; Zaliha *et al.*, 2004). Fractionation of rice bran oil was also being studied and reported in works of literature (Bakota *et al.*, 2013; Lee *et al.*, 2007; Yu *et al.*, 2006). Rice bran oil was reported to be fractionated with the aid of acetone under a temperature below 0°C. Bakota *et al.* (2014) showed that rice bran oil spreads, a product from solvent fractionation of RBO, had been successfully incorporated into baked goods with high

acceptability in a sensory test. Other forms of rice bran oil spread reported to be used as an additive in cake, doughnuts, cookies, muffins, and piecrusts (Shaik *et al.*, 2018).

In Malaysia, rice bran is considered as underutilized as the lipids in crude rice bran are easily decomposed due to lipase activity (Shafie & Norhaizan, 2017). This causes rice bran products to be less interested in consumers. As mentioned, rice bran oil spread can be used as an alternate shortening in bakery products. Shortening is usually used to coat the gluten strands and reduce a rigid matrix in bread. It helps to shorten the gluten, creating a tender bread product (Jones, 2016). However, shortening contains 100% of fat, neither protein nor carbohydrates. Besides, shortening is made by hydrogenation of vegetable oil, producing run-flat (Bussing, 2019; Jones, 2016). In contrast with rice bran oil, trans-fat in shortening helps raise low-density lipoprotein (LDL) cholesterol, increasing the risk of getting cardiovascular diseases (Iqbal, 2014).

This study aims to study the characterization of rice bran oil spread compared to commercial shortening and rice bran oil. The evaluated characteristics included physicochemical, textural, and thermal properties before incorporating rice bran oil spread in the baked goods.

2. Materials and Methods

2.1. Samples and Reagents

Commercial rice bran oil (RBO) was purchased from Greenapple Organic Sdn. Bhd, imported from India. Farmland commercial shortening (CS) that 360 Horizon Sdn Bhd manufactures purchased from Tesco Extra, Gelugor, Pulau Pinang. Stigmasterol, campesterol, and β-sitosterol were obtained from Merck (Germany). All other solvents and chemicals were purchased from R&M chemicals unless otherwise stated and were of HPLC grade or AR grade for solvents.

2.2. Solvent Fractionation of Rice Bran Oil (RBO)

Solvent fractionation of rice bran oil was carried according to Bakota *et al.* (2014). RBO was brought to room temperature before fractionation. Acetone was chilled in the freezer at -20°C for two hours before fractionation in a stoppered Erlenmeyer flask. Eighty grams of RBO was added to a beaker with 200 mL acetone, and the mixture was stirred briefly. The beaker containing the mixture was stored at -20°C for 1 hour. Then, the cold slurry was filtered through Whatman No. 3 filter paper using the Buchner funnel. The slurry

was dried in a desiccator for 24 hours and scrapped to collect the final product, rice bran oil spread (RBOS).

2.3. Physicochemical Properties

2.3.1. Determination of γ-oryzanol

According to Sawadikiat and Hongsprabhas (2014), a 10mg aliquot of sample was weighed into a 10-mL volumetric flask. Hexane was added to dissolve the sample and adjusted to the volume. The absorbance of each sample was measured at the wavelength of 314 nm using a UV-Vis spectrophotometer (Model UVmini-1240, Shimadzu, Japan). The concentration of γ-oryzanol in each sample was calculated using Equation 1 as shown below:

$$\text{Conc} = \frac{\text{A} \times \text{V} \times 1{,}000}{\text{m} \times 358.9} \quad (1)$$

Where:

A = absorbance of the sample at 314 nm in hexane solution,
V = volume of hexane used in millilitre,
m = mass of sample in gram.
358.9 = mass extinction coefficient of γ-oryzanol.

2.3.2. Determination of phytosterols

Stigmasterol, campesterol, and β-sitosterol in the samples were detected and determined according to Balachandran *et al.* (2008).

One gram of sample was added in a 250-mL round-bottom flask with 100 mL 0.8 M ethanolic potassium hydroxide (KOH). The mixture was heated at 80°C for 30 min. After heating, the mixture was placed in a 250-mL separatory funnel with 100 mL of diethyl ether and shaken. The mixture was then washed three times with 50 mL of distilled water to remove the water-soluble compounds. The ether layer containing the targeted unsaponifiable compounds was concentrated using a rotary evaporator at room temperature with a pressure of 400 MPa. Each flask was flushed with nitrogen gas, and the lipid residues containing the sterols were dissolved in 1 mL absolute ethanol. The mixture was filtered through a 0.22 µm Millipore filter and stored in an amber bottle. The samples were stored in the chiller at 4°C before being injected into HPLC.

The standards and samples were injected into RP-HPLC, which consisted of an HPLC pump (Model 515, Waters, United States) and a dual absorbance detector (Model 2487, United States). A 10-µL aliquot of standard or sample was manually injected in the mobile

phase, which consisted of a mixture of methanol: water (96.5:3.5, v/v), at a flow rate of 1.2 mL/min, passing through a C18 column (Zobrax Eclipse Plus, Agilent, United States) with a length of 150 mm, particle size of 3.5 µm, and inner diameter of 4.6 mm. The sterol compounds were detected at the wavelength of 206 nm. The peak was identified by comparing the retention times of standard solutions and confirmed with characteristic spectra using the UV-Vis detector.

2.3.3. Determination of fatty acid composition

The fatty acid composition of samples was determined according to Bouarroudj *et al.* (2016) with slight modification.

A 0.5-g aliquot of sample was weighed and shaken with 5 mL of hexane and 0.5 mL of 2 N methanolic potassium hydroxide (KOH). The mixture was stirred for 30 s and centrifuged at 3,000 rpm for 5 min. Then, 100 µL of supernatant was removed and mixed with 1 mL of hexane. The mixture was filtered through a 0.22 µm Millipore filter and stored in an amber bottle. The samples were stored in the chiller at 4°C before being injected into GC-MS.

Fatty acid methyl esters (FAMEs) were analyzed on GC-MS (Model QP 2010, Shimadzu, Japan). A capillary column (BPX70, SGE Analytical Science, United Kingdom) with a length of 30 meters, an inner diameter of 0.25 mm, and film thickness of 0.25 µm was used to separate the FAMEs compounds. A 1-µL aliquot of the FAME sample was injected with an inlet temperature of 230°C. The split mode was 1:50, and the helium gas flow was 1.03 mL/min. The initial temperature of GC oven was set at 50°C, held for 2 min, a ramp in temperature of 4°C per min to 170°C held for 5 min, then a ramp in temperature of 2°C per min to 220°C held for 10 min. The solvent was cut off for 1.5 min before MS acquisition began. The transfer line from GC column to MS was set at 230°C and the source 200°C. Source fragmentation was done with a scan range of 29 m/z to 550 m/z. The peaks were identified and compared by relating them to recognized standards.

2.4. Textural Properties

2.4.1. Spreadability test

A spreadability test was carried out using a texture analyzer (Model TA XT. Plus C, Stable Micro Systems, United Kingdom) equipped with TTC Spreadability Rig and a 30-kg load cell. The spreadability accessory consists of a set of matched male and female Perspex 45° cones. Each sample was placed in a female cone and pressed down using a spatula to

eliminate air pockets. The female cone containing the sample was inserted into the female cone sample holder while the male cone was attached to the load cell. The sample was being penetrated by a male cone at the test speed of 3.0 mm/s and withdrawn at the post-test speed of 10 mm/s after the cone reached the specific penetration depth, causing the sample to be squeezed out. Force-time graphs of the samples were recorded. Each value of firmness (g) and work of shear (g·sec) were calculated by the Exponent 32 software (Stable Micro Systems, United Kingdom).

2.5. Thermal Properties

Differential scanning calorimetry (DSC) techniques and thermogravimetric analysis (TGA) were conducted to investigate the thermal properties of CS and RBOS. RBO was not included in the thermal analysis due to its liquid form. As both CS and RBOS were solid, it is comparable in their melting point and thermal stability.

2.5.1. Differential scanning calorimetry (DSC)

DSC test was carried out using the Q200 model (TA instrument, Australia) equipped with a gas controller (Model GC10, Mettler Toledo, Malaysia). The reference was an empty, hermetically sealed aluminum pan. The samples were weighed to 5 ± 1 mg and hermetically sealed in an aluminum pan. Dry nitrogen gas with 99.99% purity was purged at 50 mL/min. Heating was done at 10°C/min from -50 to 10°C. The results were analyzed using Universal V4.7A TA Instruments software.

2.5.2. Thermogravimetric analysis (TGA)

TGA was performed by using the TGA instrument (Mettler Toledo, Malaysia). Approximately 5–10 mg of sample was weighed and placed in a ceramic crucible. The reference was an empty ceramic crucible. The thermal decomposition of each sample was monitored in an airstream at 10°C/min in the temperature range between 30 to 800°C. The results were analyzed using STARe TGA Evaluation software (Mettler Toledo, Malaysia). The first derivate (DTG) was calculated.

2.6. Statistical Analysis

All the tests were performed in triplicate. Results were shown in the form of mean ± standard deviation. For the variance homogeneity of the results, a one-way analysis of variance (ANOVA) with Tukey's test was applied using SPSS Statistics ver. 27 software (IBM, United States) at the significance level $p < 0.05$.

3. Results

3.1. Physicochemical Properties

Table 1 showed the quantitation of γ-oryzanol in three samples. Analysis of variance (ANOVA) showed that there was no significant difference ($p > 0.05$) for the concentration of γ-oryzanol between RBO and RBOS. The amount of γ-oryzanol was within the range of 1,201.86-1,299.88 mg/ 100 g sample. On the other side, γ-oryzanol was not detected in CS.

Table 1. Quantitation of γ-oryzanol in each sample.

Samples[1]	**γ-oryzanol (mg/ 100 g sample)**
CS	N.D.[2]
RBO	1,299.88 ± 50.97[a, 3]
RBOS	1,201.86 ± 84.37[a]

All values are expressed as mean value ± standard deviation. [1] CS = commercial shortening; RBO = commercial rice bran oil; RBOS = rice bran oil spread. [2] N.D. represents not detected. [3] Different alphabets in the same column denoted the significance difference ($p < 0.05$).

Table 2 showed the quantitation of phytosterols in each sample. CS has the least amount of phytosterols. Campesterol and stigmasterol were detected in CS, but β-sitosterol was not detected. From the table, there were no significant differences ($p > 0.05$) for campesterol and stigmasterol but significant differences ($p < 0.05$) for β-sitosterol and total phytosterol between RBO and RBOS. Overall, RBO (247.00 ± 0.89 mg/ 100 g sample) has higher total phytosterols concentration than RBOS (184.16 ± 0.56 mg/ 100 g sample) and CS (4.17 ± 0.26 mg/ 100 g sample).

Table 2. Quantitation of phytosterols in each sample.

Samples[1]	**Phytosterols (mg/ 100 g sample)**			
	Campesterol	Stigmasterol	β-sitosterol	Total
CS	2.50 ± 0.17[b, 2]	1.66 ± 0.43[b]	N.D.[3]	4.17 ± 0.26[c]
RBO	56.94 ± 3.64[a]	31.53 ± 3.43[a]	158.53 ± 8.02[a]	247.00 ± 0.89[a]
RBOS	46.24 ± 7.97[a]	20.04 ± 2.12[a]	117.87 ± 5.30[b]	184.16 ± 0.56[b]

All values are expressed as mean value ± standard deviation. [1] CS = commercial shortening; RBO = commercial rice bran oil; RBOS = rice bran oil spread. [2] Different alphabets in the same column denoted the significance difference ($p < 0.05$). [3] N.D. represents not detected.

The fatty acid composition of each sample was shown in Table 3. From the table, the dominant fatty acids in CS were palmitic acid (51.49 ± 0.08%) and elaidic acid (34.31 ± 0.03%). Other minor fatty acid detected in CS were arachidic acid (0.28 ± 0.06%), myristic acid (0.78 ± 0.64%), stearic acid (5.05 ± 0.19%) and linoleic acid (7.04 ± 0.02%).

There were three dominant fatty acids detected in RBO and RBOS, which were palmitic acid (19.61 ± 0.08%; 28.87 ± 0.28%), oleic acid (45.25 ± 0.12%; 38.32 ± 0.33%) and linoleic acid (30.74 ± 0.24%; 25.11 ± 0.60%). Other minor fatty acids detected in RBO were linolenic acid (0.71 ± 0.01%), arachidic acid (0.84 ± 0.01%) and stearic acid (2.09 ± 0.10%). On the other hands, other minor fatty acids detected in RBOS were behenic acid (0.25 ± 0.35%), linolenic acid (0.58 ± 0.03%), lignoceric acid (1.06 ± 0.01%), arachidic acid (1.46 ± 0.03%), and stearic acid (3.92 ± 0.43%). Overall, CS has significantly ($p < 0.05$) highest amount of SFA (57.59 ± 0.28%) but significantly ($p < 0.05$) least amount of PUFA (7.04 ± 0.02%). RBO has significantly highest amount of MUFA (45.25 ± 0.11%).

Table 3. Fatty acid composition of each sample.

Fatty acid	**Carbon number**	**Group[4]**	**Relative abundance of the sample (%)[1]**		
			CS	RBO	RBOS
Myristic acid	C14:0	SFA	$0.78 \pm 0.64^{A.\ 2}$	N.D.	N.D.
Palmitic acid	C16:0	SFA	51.49 ± 0.08^{A}	19.61 ± 0.08^{C}	28.87 ± 0.28^{B}
Stearic acid	C18:0	SFA	5.05 ± 0.19^{A}	2.09 ± 0.10^{B}	3.92 ± 0.43^{A}
Oleic acid	C18:1	MUFA	N.D.[3]	45.25 ± 0.12^{A}	38.32 ± 0.33^{B}
Elaidic acid	C18:1 (trans)	MUFA	34.31 ± 0.03^{A}	N.D.	N.D.
Linoleic acid	C18:2	PUFA	7.04 ± 0.02^{C}	30.74 ± 0.24^{A}	25.11 ± 0.60^{B}
Linolenic acid	C18:3	PUFA	N.D.	0.71 ± 0.01^{A}	0.58 ± 0.03^{B}
Arachidic acid	C20:0	SFA	0.28 ± 0.06^{C}	0.84 ± 0.01^{B}	1.46 ± 0.03^{A}
Arachidonic acid	C20:4	PUFA	N.D.	N.D.	N.D.
Behenic acid	C22:0	SFA	N.D.	N.D.	0.25 ± 0.35^{A}
Erucic acid	C22:1	MUFA	N.D.	N.D.	N.D.
Lignoceric acid	C24:0	SFA	N.D.	N.D.	1.06 ± 0.01^{A}
SFA			57.59 ± 0.28^{Aa}	22.53 ± 0.18^{Cc}	35.55 ± 0.33^{Bb}
MUFA			34.31 ± 0.03^{Cb}	45.25 ± 0.11^{Aa}	38.32 ± 0.33^{Ba}
PUFA			7.04 ± 0.02^{Cc}	31.45 ± 0.23^{Ab}	25.69 ± 0.63^{Bc}

All values are expressed as mean value ± standard deviation. [1] CS = commercial shortening; RBO = commercial rice bran oil; RBOS = rice bran oil spread. [2] Different alphabets in the same column and row denoted the significance difference ($p < 0.05$). [3] N.D. represents not detected. [4] SFA: saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid.

3.2. Textural Properties

Table 4 showed the spreadability of the CS and RBOS sample. Firmness is defined as the force penetrated at the maximum depth, while shear work is a good indicator of spreadability. The work of shear, which is the area under the positive curve, is also known as

the total force amount needed to carry out the shearing process. The difference in firmness and work of shear results for both samples were not significantly ($p > 0.05$) significant. The firmness of samples was within the range of 1,226.66–1,256.32 g, while shear work was within the range of 1,022.68–1,099.60 g·sec.

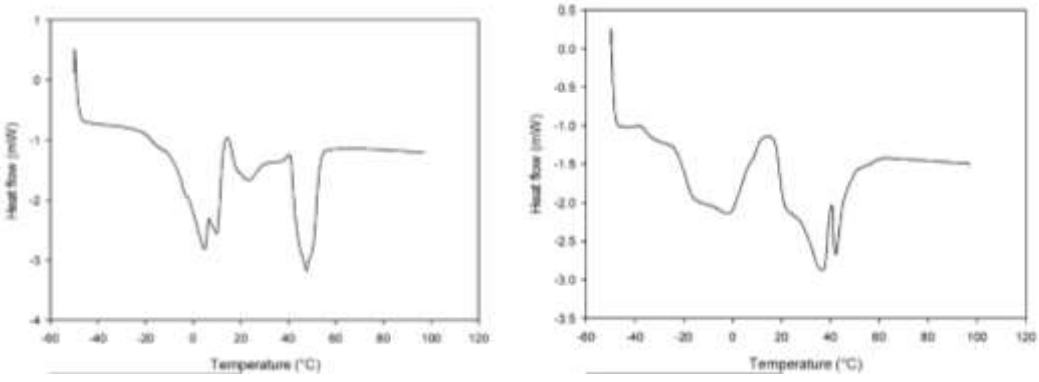
Table 4. Spreadability value of each sample.

Samples[1]	Firmness (g)	Work of shear (g·sec)
CS	1,226.66 ± 44.03[a, 2]	1,022.68 ± 18.72[a]
RBOS	1,256.32 ± 127.98[a]	1,099.60 ± 82.00[a]

All values are expressed as mean value ± standard deviation. [1] CS = commercial shortening; RBOS = rice bran oil spread. [2] Same alphabets in the same column denoted the non-significance difference ($p > 0.05$).

3.3. Thermal Properties

DSC thermogram of CS and RBOS were shown in Figure 1. Mild and wide endothermic peaks were observed in both CS and RBOS melting curves. The melting curve of CS was started approximately from -8.43°C to 55.42°C while the melting curve of RBOS was in the range of temperature from -23.08°C to 50.76°C. In the thermogram of CS, there were three sharp peaks observed at the melting curve, which were approximately 4.90°C, 9.91°C, and 47.76°C. On the contrary, for the RBOS, one sharp peak associated with few irregulars, shoulder endotherms were noticed at the melting curve. The sharp peak was at the maximum temperature of 42.25°C. The difference in melting enthalpy for both samples was significantly ($p < 0.05$) significant.



(a) **(b)**

Figure 1. DSC thermogram: **(a)** CS; **(b)** RBOS.

Table 5. Melting profile of CS and RBOS.

Samples[1]	T_{on}[2] (°C)	T_{off} (°C)	T_r (°C)	T_p (°C)	ΔH (J/g)
CS	-8.43	55.42	63.85	47.76	44.65
RBOS	-23.08	50.76	73.84	42.45	57.91

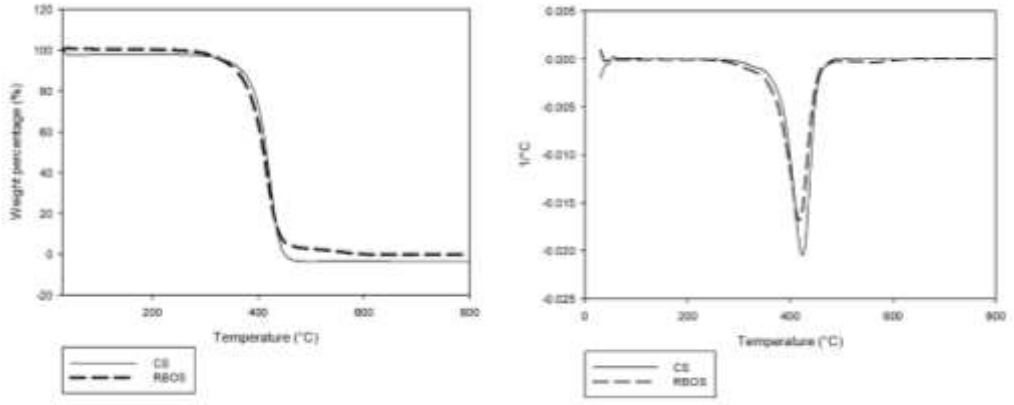
[1] CS = commercial shortening; RBOS = rice bran oil spread. [2] T_{on}: onset temperature; T_{off}: offset temperature; T_r: temperature range; T_p: peak temperature; ΔH: melting enthalpy.

On the other hand, tested temperatures of CS and RBOS were shown in Table 6. The difference in onset and peak temperature results in both samples were not significant (p > 0.05) important. The onset temperature of samples was within the range of 389.02–393.20°C, while the peak temperature was within the range of 420.23–421.01°C. Single-phase decomposition was observed in Figure 2.

Table 6. The temperature of each sample in TGA.

Samples[1]	T_{on}[3] (°C)	T_{peak} (°C)
CS	393.20 ± 0.05[a, 2]	421.01 ± 1.05[a]
RBOS	389.02 ± 5.35[a]	420.23 ± 2.75[a]

All values are expressed as mean value ± standard deviation. [1] CS = commercial shortening; RBOS = rice bran oil spread. [2] Same alphabets in the same column denoted the non-significance difference (p > 0.05). [3] T_{on}: onset temperature; T_{peak}: peak temperature.



(a) **(b)**

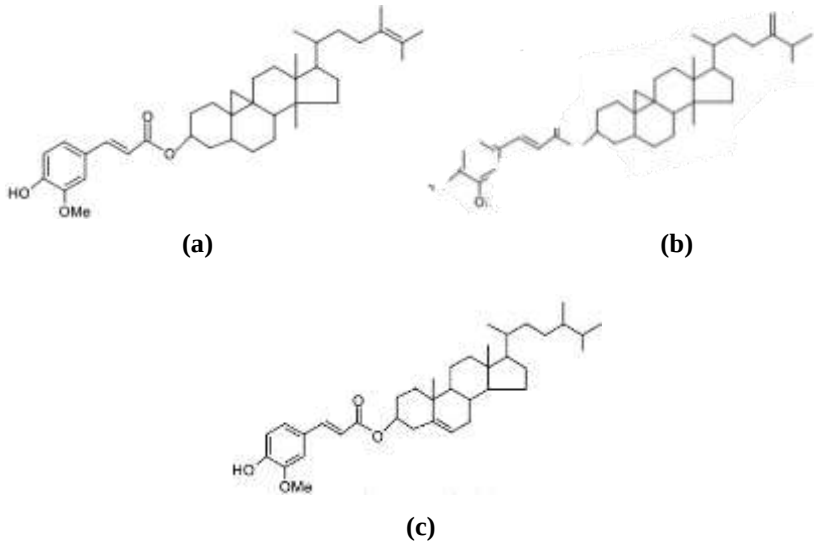
Figure 2. (a) TGA thermogram; **(b)** DTG graph for CS and RBOS. The solid line is represented as CS while the dotted line is represented as RBOS.

4. Discussion

4.1. Physicochemical Properties

The γ-oryzanol is a distinct compound that physiologically present in rice (*Oryza sativa*). Oryzanol is not a single compound but a mixture including ferulic acid esters, such

as β-sitosterol ferulate, campesteryl ferulate, cycloartenyl ferulate, 24-methylene cycloartenol ferulate, and others (Figure 3) (Patel & Naik, 2004; Srikaeo, 2014). Oryzanol helps decrease plasma cholesterol levels (Francisqueti *et al.*, 2018; Wang *et al.*, 2015) and aggregation of platelet (Wong *et al.*, 2016). Besides, it shows antioxidant properties by scavenging free radicals and control inflammatory diseases (Panchal *et al.*, 2017; Xu & Godber, 2001).



(a) **(b)**

(c)

Figure 3. Dominant components of γ-oryzanol (Srikaeo, 2014): **(a)** Cycloartenyl ferulate; **(b)** 24-Methylene cycloartanyl ferulate; **(c)** Campesteryl ferulate.

In the present study, UV-spectrophotometry was used to detect total γ-oryzanol content due to its simplicity and shorter testing period compared to HPLC (Bhatnagar *et al.*, 2014; Pungseeklao *et al.*, 2016). On the other hand, γ-oryzanol in RBO and RBOS was detected in the range of 1,201.86-1,299.88 mg/ 100 g sample. This result is in accordance with other studies (Heidtmann-Bemvenuti *et al.*, 2012; Khatoon & Gopalakrishna, 2004; Sawadikiat & Hongsprabhas, 2014).

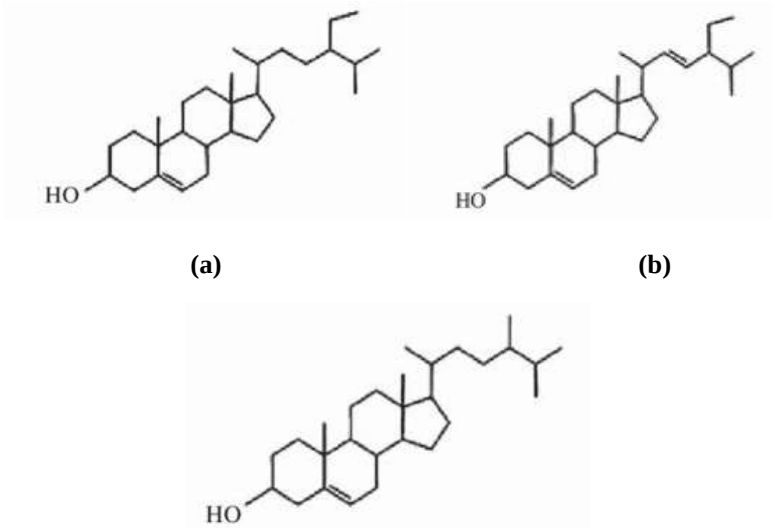
Table 7. Detection and quantitation of γ-oryzanol in rice bran and its oil using UV-Vis spectrophotometer.

Sample	**Concentration (mg/ 100g sample)**	**Reference**
Rice bran	150–1,398	(Heidtmann-Bemvenuti *et al.*, 2012)
Rice bran oil	Brown rice: 1,070–1,430 Milled rice: 450–630	(Khatoon & Gopalakrishna, 2004)
Rice bran oil	304.43–716.46	(Pungseeklao *et al.*, 2016)
Rice bran oil	Crude oil: 1,599–1,666 Refined oil: 933–960	(Sawadikiat & Hongsprabhas, 2014)

On the contrary, most γ-oryzanol and phytosterol compounds could not be found in CS because they are made from 100% fat and contain very few bioactive compounds (Jones, 2016).

Similar to γ-oryzanol, phytosterols also help reduce serum cholesterol concentration in the human body (Özdestan *et al.*, 2014; Vissers *et al.*, 2000; Tolve *et al.*, 2020). Campesterol, stigmasterol, and β-sitosterol are the dominant phytosterols in rice bran (Figure 4). The current study found that the detected phytosterol in RBO and RBOS were lesser than those recorded in other literature works (Table 8). Commonly, phytosterols were detected and measured by gas chromatography (GC). Nevertheless, the preparation of samples before GC injection includes saponification, extraction, derivatization, and time-consuming others (Lagarda *et al.*, 2006; Plante *et al.*, 2011). Hence, a more straightforward HPLC method was used in the study.

Similar to unsaturated fatty acids and cholesterol, phytosterols are easy to be oxidized due to the presence of steroid rings in phytosterols that are susceptible to autoxidation (Lin *et al.*, 2019; Tolve *et al.*, 2020). Therefore, it was believed that some phytosterols were oxidized during the sample preparation that contributed to the low detected concentration.



(a) **(b)**

(c)

Figure 4. Major phytosterols in rice bran oil (Vaquero *et al.*, 2010): **(a)** β-sitosterol; **(b)** stigmasterol; **(c)** campesterol.

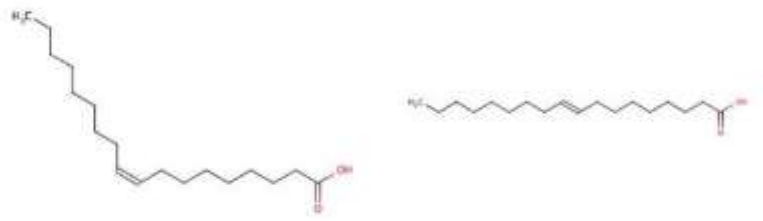
Table 8. Detection and quantification of phytosterol in rice bran and its oil

Sample	Detection method	Concentration (mg/ 100g sample)	Reference
Rice bran oil	RP-HPLC	Stigmasterol: 182–270 β-Sitosterol: 1020–1270 Campesterol: 400–490	(Balachandran *et al.*, 2008)
Rice bran	GC-MS	β-Sitosterol: 128.00–133.07 Campesterol: 69.61–75.68	(Derakhshan-Honarparvar *et al.*, 2010)
Rice bran oil	GC-FID	**Crude oil:** Stigmasterol: 235–246 β-Sitosterol: 839–846 Campesterol: 281–292 **Refined oil:** Stigmasterol: 126–136 β-Sitosterol: 520–576 Campesterol: 164–193	(Sawadikiat & Hongsprabhas, 2014)
Rice bran oil	GC-MS	Stigmasterol: 177–205 β-Sitosterol: 497–625 Campesterol: 203–352	(Mingyai *et al.*, 2018)
Rice bran oil	GC-MS	Stigmasterol: 221.2 β-Sitosterol: 735.7 Campesterol: 226.4	(Yang, et al., 2019)

RP-HPLC: reverse-phase high-performance liquid chromatography; GC: gas chromatography; FID: flame ionization detector; MS: mass spectrometry. (Lagarda *et al.*, 2006)

RBO has a balanced fatty acid composition for fatty acid composition compared to other vegetable oils, as it was detected to contain 22.53% SFA, 45.25% MUFA, and 31.45% PUFA in the current study. The dominant fatty acid found in RBO were palmitic acid, oleic acid, and linoleic acid. These results agreed with many studies (Akhter *et al.*, 2016; Balachandran *et al.*, 2008; Latha & Nasirullah, 2014; Mas'ud *et al.*, 2017). As the end product of solvent fractionation of RBO, the fatty composition of RBOS was similar to RBO. However, the relative abundance of SFA was observed to be higher in RBOS when compared to RBO. Besides, the unsaturated fatty acid was found to be lower in RBOS. The increasing of SFA content increased the melting point, made the RBOS exist in solid form. The primary fatty acids in CS were palmitic acid (51.49%) and elaidic acid (34.31%). These results were in agreement with those from the study of El-Gawad *et al.* (2015). As a product of partial

hydrogenation of palm oil, CS has a similar fatty acid composition (Montoya *et al.*, 2014), unless elaidic acid. Elaidic acid is the trans-isomer of oleic acid (Figure 5).



(a) **(b)**

Figure 5. (a) oleic acid; **(b)** elaidic acid

RBO and RBOS were rich in MUFA and PUFA. MUFA helps to increase HDL cholesterol and reduce the probability of getting cardiovascular diseases. Diabetes mellitus can be controlled by reducing the glycosylated hemoglobin by MUFA (Salgado *et al.*, 2019). PUFA has been associated with somebody's system functioning, such as cardiovascular, endocrine, immune, inflammatory, nervous, reproductive, respiratory, and others (Srikaeo, 2014). With their presence, cellular activities are affected, and membrane fluidity is altered. These will result in the modulation of enzymes, receptors, and transporters' actions (Srikaeo, 2014). In addition, α-linoleic acid and linoleic acid are essential fatty acids since the human body cannot synthesize them but only can be taken from the human diet (Salgado *et al.*, 2019). Albeit high SFA content in RBO compared to other vegetable oils, the unsaponifiable matter in RBO is also responsible for reducing cholesterol level (Chandrashekar *et al.*, 2014; Zavoshy *et al.*, 2012).

Table 9. Fatty acid composition of common vegetable oils.

Oil type	Fatty acid			Reference
	SFA	**MUFA**	**PUFA**	
Coconut oil	92.1	6.2	1.6	(Orsavova *et al.*, 2015)
Corn oil	19.0-23.0	19.0-50.0	34.0-64.0	(List, 2016)
Canola oil	7.0	50.0-65.0	6.0-14.0	(Nandasiri *et al.*, 2020)
Peanut oil	10.7	71.1	18.2	(Orsavova *et al.*, 2015)
Palm oil	41.0-51.4	27.2-50.0	7.1-26.0	(Montoya *et al.*, 2014)

Oil type	Fatty acid			Reference
	SFA	MUFA	PUFA	
Rapeseed oil	6.3	72.8	20.9	(Orsavova *et al.*, 2015)
Sunflower oil	7.7-13.6	14.0-39.4	48.3-74.0	(Akkaya, 2018)
Soybean oil	10.4-18.7	17.7-26.1	55.3-66.6	(List, 2016)
Safflower oil	9.3	11.6	79.1	(Orsavova *et al.*, 2015)
Olive oil	15.0-20.5	58.8-72.4	10.9-20.6	(Riachy *et al.*, 2019)

SFA: saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid.

4.2. Textural Properties

Spreadability is an essential attribute of spreadable products like cream, butter, spread, margarine, and others. It is known as the pressure needed so that the spreadable products can be uniformly distributed over a surface (Bayarri *et al.*, 2012). The difference in firmness and work of shear results in CS and RBOS were not significantly ($p > 0.05$) significant. The lower the firmness and work of shear, the more spreadable the product (Bayarri *et al.*, 2012). Therefore, it is concluded that RBOS has demonstrated similar spreadability with CS. The fat crystal networks associated with the liquid oil phase bring to the spreadability, as the ratio of solid to liquid fat determines the consistency of spreads and shortening. Apart from solid fat content, the hardness of a spreadable product is also evaluated by crystal size and polymorphic behaviour (Glibowski *et al.*, 2008). A spreadable product requires to fulfill a few criteria (Carr & Vaisey-Genser, 2003). It must be able to stable and maintain its form at room temperature. Besides, it can spread quickly over a broad temperature range. Moreover, it must melt and release the flavor in the mouth in a short time without any gummy sensation (Carr & Vaisey-Genser, 2003).

4.3. Thermal Properties

Both CS and RBOS have a broad melting temperature range, as they possess mixtures of triglyceride esters with varied unsaturation (Omar *et al.*, 2017; Wang *et al.*, 2017). CS, palm oil-based, has a melting point within the range of 34–44°C since palm oil has a higher melting point than other common vegetable oils (Lai *et al.*, 2012). RBOS has a significantly ($p < 0.05$) lower melting point and enthalpy than CS due to significantly ($p < 0.05$) lower SFA content. The lower the enthalpy, the lower energy required to melt RBOS compared to CS (Devi & Khatkar, 2017).

According to Devi and Khatkar (2017), the first endotherm region represented the melting process of α polymorph while the second endotherm region corresponds to the melting of β' polymorph. Besides, the melting of β polymorph is represented by the last endotherm region. According to Dolatowska-Zebrowska *et al.* (2019), a smaller shoulder peak at the melting curve represents the melting of trans-configurated monounsaturated fatty acid and long-chain saturated fatty acid with carbon numbers 16 and above. On the contrary, the melting of unsaturated fatty acid and fatty acid with carbon numbers below 16 contributes to the large shoulder peak formation (Dolatowska-Zebrowska *et al.*, 2019).

For the TGA test, the difference in onset and peak temperature results in both samples were not significant ($p > 0.05$) important. Both CS and RBOS have only single-phase decomposition. CS showed more excellent thermal stability than RBOS but not significantly ($p > 0.05$) different.

5. Conclusions

In the present study, RBOS contains a higher amount of bioactive compounds such as γ-oryzanol and phytosterols that indicate better physicochemical properties than CS. RBOS and RBO have balanced fatty acid compositions, which makes them beneficial and functional. RBOS has similar textural properties and decomposition temperature to CS. Although RBOS has a lower melting point than CS, it still stables at room temperature. The results of this study are a promising indication that RBOS could be a suitable option as an alternate shortening in bakery products.

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