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Development of Rice Bran Oil Incorporated Functional Margarine and Assessing Its Physicochemical and Sensory Properties

A.A. Edirisooriya^{1*}, O.C.P. De Silva¹, S. Somasiri², T. Madhujith³

¹ Food Science and Technology, Naratha Agro Industries Pvt. Ltd, Nugape, 11328, Sri Lanka

² Industrial Technology Institute, Colombo 7, Sri Lanka

³ Department of Food Science and Technology, Faculty of Agriculture, University of 10 Peradeniya, Sri Lanka

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*Corresponding author:

anujaniae22@gmail.com

ABSTRACT

Background: Rice (*Oryza sativa*), a staple food in Asia, produces rice bran rich in protein, vitamins, and oil (18–22%). The γ -oryzanols in rice bran oil (RBO) are noted for their antioxidant properties and a balanced fatty acid profile.

Objectives: This study aimed to develop a heart-healthier margarine for Sri Lanka by incorporating different ratios of RBO (5%, 10%, 15%, 20%) by replacing the current saturated fat content.

Material & Methods: Rice bran samples were collected from North and North central provinces of Sri Lanka and rice bran oil was extracted using soxhlet extraction with n-hexane as the solvent. Different levels of rice bran oil (5, 10, 15 and 20%) were mixed with coconut oil to make margarine. Physico-chemical parameters, oxidative stability and sensory properties of the margarine samples were evaluated.

Results: The results indicated that 20% RBO significantly increased oleic, linoleic, and α -linolenic acids, decreased saturated fat ($42.61\% \pm 0.06$), and increased monounsaturated ($22.40\% \pm 0.02$) and polyunsaturated ($10.34\% \pm 0.01$) fats. Four antioxidant blends were formulated with 0.015% Ascorbyl palmitate (AP) and TBHQ (tert-Butylhydroquinone), 0.2% rosemary extract, and 20% RBO with 0.0075% AP which was prepared considering RBO as a natural antioxidant. The oxidative stability of RBO blends (5%, 15%, and 20%) and γ -oryzanol content of RBO blends (10%, 15%, and 20%) showed no significant differences ($P > 0.05$). The final margarine, containing 20% RBO and 0.0075% AP, exhibited lower oxidative stability compared to TBHQ but similar to 0.015% AP.

Conclusion: Despite not being suitable for commercial bakery use due to its spreadability at room temperature, this margarine demonstrates that RBO can be effectively used in margarine manufacturing, offering a functional food option that supports heart health.



INTRODUCTION

Margarine is a water-in-oil (W/O) emulsion prepared by blending vegetable oils. Most margarines are made from edible oils such as palm oil and coconut oil rich in saturated fat (SAF) which are associated with the risk of coronary heart diseases (Sivakanthan et al., 2023). Therefore, modification of the fatty acid profile of margarine by incorporating/blending favorable oils is beneficial. Rice bran oil (RBO) is extracted from the rice bran, with a fatty acid profile closer to WHO (World Health Organization) recommendations (Sivakanthan et al., 2023, p. 108839). There is a growing interest in RBO due to the health and nutritional properties of the unique bioactive compounds, especially γ -oryzanol. Various studies have found that oryzanol claims to have hypocholesterolemic effects and to have a protective role in lipid oxidation and thus has potential as an antioxidant (Hariri et al., 2023). Commercially, lipid oxidation is still a major problem during storage despite the wide use of synthetic antioxidants. Since there are some serious problems concerning the safety and toxicity of synthetic antioxidants such as BHA (Butylated hydroxy anisole), BHT (Butylated hydroxytoluene), and TBHQ (tert-butylhydroquinone) related to their metabolism (Wang et al., 2021), there is a potential for useful natural antioxidants which lack safety issues. Apart from oryzanol, rice bran oil is surprisingly rich in other bioactive constituents such as tocopherols, phytosterols, polyphenols, and squalene which also accounts for antioxidative properties (Yeon et al., 2020). Therefore, since RBO and its use in blending has been found to improve the stability of blends for frying and storage (Hariri et al., 2023), it can be used as a natural antioxidant to extend shelf life and improve the nutritional status of margarine.

Even if, 2.9 million tons of rice is produced annually in Sri Lanka, rice bran is not utilized for human consumption (Department of Census and Statistics, 2021). Almost all rice bran is utilized to produce animal feeds currently in Sri Lanka. The main objective of this study was to develop functional margarine by incorporating

RBO as a substitute for conventional oils and to compare the physicochemical and sensory properties of margarine. In addition, this aims to enhance the nutritional value of margarine by modifying its fatty acid profile, evaluate the effectiveness of RBO/oryzanol as a natural antioxidant in preventing lipid oxidation, and compare its antioxidant properties with those of synthetic antioxidants to ensure the quality and stability of the developed margarine. The results of this work will serve as useful information for further application of RBO as a potential for food and pharmaceutical industries and to draw the attention of researchers and the public about RBO and its intended uses.

MATERIALS & METHODS

Sample collection

Rice bran from red and parboiled rice was collected from the millers in the North Central and North Western provinces of Sri Lanka and harvested in the Maha season (October- March 2021). From March to April 2021, rice bran samples were collected just after polishing and about 5 kg of bran was collected from each miller. Samples were packaged in sealed polyethylene bags and transported to the factory premises of Naratha Agro Industries Pvt. Ltd. Refined palm oil (RPO), refined coconut oil (RCNO), and refined palm stearin (PS) were obtained from Naratha Agro Industries, Nugape, Sri Lanka. TBHQ and Ascorbyl Palmitate (AP) were purchased from Camlin Fine Science Ltd-India, Yasho Industries-India, respectively and Rosemary extract was purchased from Naturex, France. The approved color (Annato red) and approved flavor (nature identical butter flavor) were purchased from Chr. Hansen Horsholm, Denmark, and Givaudan, Geneva, Switzerland respectively. Potassium sorbate, Phosphoric acid, and Calcium Oxide were purchased from Jenning Science and Technology, China, and free-flow iodized salt was purchased from Raigam Wayamba Salterns, Sri Lanka. Lecithin was purchased from Cargill, USA. Commercial margarine 1 (CM1) was purchased from Marina

Foods Pvt. Ltd and Commercial margarine 2 (CM2) and competitor table margarine were purchased from Pyramid Wilmar (Pvt.) Ltd., Sri Lanka.

Sample preparation

Preparation of rice bran

The collected rice bran was sieved through a 150 µm sieve to remove extraneous matter. Then, the remainder was stabilized using a domestic microwave oven at medium power level (National, S327WF, Japan) for 3-5 minutes and cooled to room temperature as explained by Reis et al. (2021). The aforesaid procedure was repeated three times, and the stabilized rice bran was transferred to vacuum packaging and stored in the freezer at -20 °C for further use.

Extraction of rice bran oil

Rice bran oil was extracted using solvent extraction with n-hexane as the solvent using Soxhlet apparatus. The process was carried out using a water bath at 50 °C for 8-9 hours with 300 mL of n-hexane. The solvent was distilled off in *vacuo* at 45 °C. The oil extraction rate was calculated and extracted RBO was dewaxed, degummed, physically refined for edible purposes, and stored at -20 °C until used.

Degumming

Degumming and dewaxing were done according to the process given by De and Bhattacharya, (1998). The extracted crude rice bran oil was heated to 65 °C and mixed with 5% (w/w) water or 0.2% phosphoric acid as 10% solution in water and stirred for 1 hour. Then, the clear upper layer was decanted after the oil was centrifuged at 10,000 rpm for 15 min. After that, the acid was neutralized by adding Calcium Oxide and centrifuged. Finally, the oil was washed with hot water and was added cooled to 20 °C with mild stirring.

Dewaxing

The stirring continued for 1 hour and then the

oil was centrifuged at 10,000 rpm for 15 min at 20 °C. The oil was decanted and dried under a vacuum of 30 mmHg pressure and at 90 °C.

Refining

Oils were refined using the physical refining method which had been bleached and deodorized. Bleaching was performed by heating oil to 50-55 °C and the oils were filtered through beaching earth (Bajaj, India) followed by (12 µm) pressure leaf filter (NYB 12, Yixing machinery, China) and (10µm) polishing filter (PTSWCD-508/1500, Mahle, Netherlands). The deodor process was carried out under vacuum (0.5-8 mbar) and at a temperature of 180-270 °C with the stripping steam of 0.50%–2% (w/w). Then the filtered oils were transferred to relevant storage tanks.

Analysis of raw materials

Before preparation of the margarine blend, physicochemical properties such as color, relative density, refractive index (RI), the content of saponifiable matter (SAP), free fatty acid content (FFA) peroxide value (PV), and slip melting point (SMP) of RCNO, RPO RBO, and PS were analyzed and compared according to SLS (Sri Lanka Standards) (SLS32, 2017), (SLS720, 2016) and (SLS277, 1987) and (SLS1427, 2011).

Determination of color

The color of the oils was determined according to AOCS (American Oil Chemists' Society)13b-45 using a Lovibond tintometer in a 1-inch cell (2.54 cm) on the Lovibond scale and expressed as 5R+Y units. Palm oil, RBO, and melted PS were measured using a 5.25-inch cell (AOCS, 2017).

Determination of refractive index

The refractive index of oils and PS was measured as explained in AOCS Cc 7-25 where samples were melted and filtered. The refractometer (R5, Mettler Toledo, USA) was adjusted for 40 °C (60 °C for 155 palm stearin) and after placing several drops of sample on the

pre-cleaned and dried prism, it was closed tightly and allowed to stand for 1-2 min until sample comes to instrument temperature. Then, after adjusting the instrument the most distinct reading was taken and a triplicate analysis was performed (AOCS, 2017).

Determination of SMP

The slip melting point of palm stearin and oil blends was measured using the capillary tube method explained in AOCS Cc 3.25 by filling 1cm of fat into a capillary tube after chilling at 10 ± 1 °C for 16 hours (AOCS, 2017).

Determination of relative density

Oils and melted palm stearin samples were filled to dry and pre-weighed specific gravity bottle and kept in a water bath for 30 ± 0.20 °C for 30 mins for oils and 90 ± 0.20 °C for 30 mins for palm stearin as explained in AOCS Cc 10c-95. After that, the bottle was removed from the bath and cleaned, dried, and weighed once it cooled to room temperature (AOCS, 2017).

Determination of saponifiable matter content

As explained in AOCS Ca 5a-40, oils and palm stearin were melted and filtered through filter paper. Then, about 2.00 ± 0.01 g of oil was measured into a conical flask 25 ml of the alcoholic KOH solution was added and a reflux air condenser was connected. Then it was boiled for more than 1 hour in a water bath until the sample was completely saponified. Once the content was cooled, it was washed by adding 10 ml of hot ethyl alcohol. Then the mixture was titrated with 0.5N HCl solution with a Phenolphthalein indicator (AOCS, 2017).

Determination of free fatty acid content

FFA content of raw materials and fat blends was quantified according to method AOCS Ca 5a-40 (AOCS, 2017). Pre-weighed samples of melted oil (12 ± 0.01 g wb) were taken into a 250 ml flask and pre-weighed (25 ± 0.01 ml wb) pre-neutralized ethyl alcohol and 1 ± 0.01 ml

phenolphthalein was added. After boiling gently for some minutes, it was titrated against 0.1 N aqueous sodium hydroxide by shaking vigorously until the first appearance of the persistent pink color of the same intensity of neutralized alcohol before sample addition.

Determination of peroxide value (PV)

PV of raw materials and fat blends were measured using AOCS Cd 8-53 by melting pre-weighed samples of oils (5 ± 0.01 g wb) and adding 30 ml of 3:2 (v/v) acetic acid: chloroform solution swirled to dissolve the sample. Then (0.5 ± 0.01 g wb) of saturated potassium iodide was added and after shaking for 1min, 30 ± 0.01 ml distilled water was added and titrated with 0.01 N sodium thiosulphate until the yellow color disappeared, using starch indicator (AOCS, 1997).

Preparation of oil blends

Generally, margarine is produced by mixing an oil phase (oil and fat-soluble ingredients) and an aqueous phase (water and water-soluble ingredients) with relevant process parameters, the required W/O emulsion was prepared. Therefore, initially, different oil combinations were prepared using palm stearin and oil with acceptable chemical and physical parameters. The oil combinations were then chemically analyzed, fat-soluble ingredients were added, mixed with an aqueous phase, and then processed as an emulsion/margarine. The prepared oil combinations are referred to as "oil blends" in this article. First, the palm stearin was melted to 85 °C, and coconut oil, palm oil, and RBO were heated to 60 °C in a water bath. Then, the mixture was agitated using a high shear agitator (RO 122, Remi Motor, India) at a slow speed of 20-25 rpm for 10 minutes until the oils were mixed properly. The filtered oil fraction was taken for further analysis.

Preparation of standard oil blend

The standard oil blend (SOB) was prepared by mixing 33% palm oil, 44% coconut oil and 4% palm stearin where the total oil fraction accounts for 81% of the total weight taken to

prepare the standard margarine (control sample).

Preparation of RBO blends

Initially, novel oil blends were prepared by adding different quantities of RBO (5, 10, 15, and 20%) to the standard oil blend as described above by replacing coconut oil content. Secondly, with the sole objective of comparing the oxidative stability of novel blends and assessing the natural antioxidant capacity of RBO another 3 blends were prepared with

chemically analyzed SOB by incorporating commonly used antioxidants in the industry which are AP (0.015%), TBHQ (0.015%) and rosemary extract (0.2%). The Final blend was prepared with chemically analyzed Blend 4 adding AP (0.0075%) (half of the standard AP amount for margarine) and RBO (20%). Therefore, these 4 blends were only subjected to the measurement of oxidative stability. The amounts of additives were based on SLS277, 1987, and SLS1427, 2011. The formulations are mentioned in the following Table 1.

Table 1. Formulation of novel RBO blends

Blend	RBO (%)	PO (%)	RCNO (%)	PS (%)	AP (%)	TBHQ (%)	Rosemary extract (%)
SOB	-	33	44	4	-	-	-
Blend 1	5	33	39	4	-	-	-
Blend 2	10	33	34	4	-	-	-
Blend 3	15	33	29	4	-	-	-
Blend 4	20	33	24	4	-	-	-
Blend 5	-	33	44	4	0.015	-	-
Blend 6	-	33	44	4	-	0.015	-
Blend 7	-	33	44	4	-	-	0.2
Blend 8	20	33	24	4	0.0075	-	-

RBO = Rice bran oil, RCNO = Refined coconut oil, PS = Refined palm stearin, AP = Ascorbyl Palmitate, TBHQ = Tert-butylhydroquinone

Analysis of blends

Novel blends were analyzed for FFA amount, PV, and SMP by the above-mentioned methodology and verified for the accepted range for novel margarine preparation according to SLS 277, 1987.

Measurement of solid fat content

The solid fat content of the sample blends was measured using pulsed NMR (mq20, minispec, Bruker, Germany) using AOCS Cd 16-81 (AOCS, 2017). The equipment was calibrated with three supplier-certified SFC standards (0%, 30%, and 70%). Samples were pipetted into p-NMR tubes. Each blend was melted at 80 °C for 15 min and then subjected to 60 °C for 10 min, followed by 0 °C for 60 min. Finally,

each sample was kept for 30 min at each measuring temperature before analysis. The SFC was measured at 10 °C intervals from 10 °C to 40 °C.

Determination of fatty acid composition

Oil blends were converted to their fatty acid methyl esters (FAME) as explained in AOAC (Association of Official Agricultural Chemists) 969.33 (AOAC, 2000). Sample (50 mg) of oil blends were mixed with methanolic NaOH and heated at 100 °C in the water bath for 5 min and cooled to room temperature. Then, BF₃ methanol (2 mL) was added to the sample and heated at 100 °C for 3 min. Then 2-5 ml of n-Heptane was added to the sample and was boiled for another 1 min followed by cooling again to room temperature and (1 mL) of

saturated NaCl was added. After shaking the mixture vigorously, 1 mL of the upper heptane layer was transferred to a vial. Aliquots (1 μ L) of the extracts were injected into a gas chromatograph (GC- 2010 pro, Shimadzu, Japan) equipped with an auto-injector, a flame ionization detector, and a fused-silica capillary column (Restek Rt- 2560, 100 m $\times$ 0.25 mm I.D, Restek, Bellefonte, PA, USA). The temperature of the injector and detector was set at 240 and 250 $^{\circ}$ C, respectively. The column was kept at 120 $^{\circ}$ C for 3 min, then heated to 200 $^{\circ}$ C at a rate of 10 $^{\circ}$ C min $^{-1}$ and kept for another 30 min at the final temperature. Helium was used as the carrier gas, and the total gas flow rate at the inlet was 30 mL min $^{-1}$. The Fatty acid composition was identified by comparison with the retention times of standard mixtures provided by Restek, Bellefonte, PA, USA. Triplicate analyses were performed.

Determination of γ -oryzanol content

Oryzanol content of crude oil, dewaxed oil, refined oil, and oil blends was determined by dissolving 0.01 ml of the sample in 10 ml of hexane and reading the absorbance at 314 nm in a 1-cm cell (Chemito UV-VIS-2100 spectrophotometer, India) (Lv et al., 2023), (Bucci et al., 2003) (Krishna et al., 2006). The oryzanol content was calculated using the following equation.

$$\gamma\text{-oryzanol}\% = [(A/W) \times (100/358.9)] \quad (1)$$

Where, A absorbance of the sample, W = weight of the sample in gram/100 ml, and 358.9 = specific extinction coefficient for oryzanol.

Measurement of oxidative stability

The oxidative stability of the above oil blends was determined by an accelerated study done by Rancimat (743, Metrohm, Switzerland) at 140 $^{\circ}$ C according to AOCS Cd 12b-92 (AOCS, 2017). The stability of the blends was evaluated based on mean induction time. Pre-weighted (3 ± 0.01 g wb) samples of each oil blend were

transferred into reaction vessels. The rate of airflow through the sample was 20 L/h. The volatile components formed were collected into a glass vessel containing 60 mL of distilled water. The resulting curves due to by-products formed by secondary oxidation were evaluated and the oxidative stability index was extrapolated at 25 $^{\circ}$ C for each sample automatically by the software in the equipment.

Preparation of margarine

Based on the results of all the above measurements, the optimum RBO blend to develop the novel margarine was determined. The other fat-soluble additives were added to the novel RBO blend to produce the complete oil phase by melting at 80 $^{\circ}$ C along with mild agitation. After holding the prepared oil phase for 10 mins at 80 $^{\circ}$ C, the temperature was reduced and maintained at 45 $^{\circ}$ C. Then the premixed aqueous phase at room temperature was slowly added to it with a speed of 500 rpm for 10 mins by a high shear agitator (RO 122, Remi Motor, India). Then the mixture was transferred to a holding tank and further agitated for 7 mins at a speed of 12000 rpm to form a fine emulsion. Then it was cooled to 9-12 $^{\circ}$ C (~ 12 $^{\circ}$ C) at a rate of ~ 6 $^{\circ}$ C min $^{-1}$ by a scraped surface heat exchanger (Sinitator, Precision Engineering Limited, Bangalore, India) with a constant shear rate of 100 rpm. The crystallized margarine emulsion was filled into 250 g polyethylene tubs and stored in the freezer (-18 $^{\circ}$ C) for 48 hours to allow complete crystallization. The percentages of the ingredients of novel and standard margarine are presented in Table 2.

Characterization of whipping properties of the novel margarine

Whipping properties were determined based on the measurement of specific gravity as a function of time. Pre-weighted samples of (350 ± 0.01 g wb) of novel margarine, standard margarine, and two commercial margarine samples exclusively intended for baking were

Table 2. Percentages of ingredients of novel and standard margarine blends

Additive	Amount (%)		
	Standard Margarine	Novel Margarine	
RBO	-	20	
Palm Oil	33	33	
Coconut oil	44	24	
Palm stearin	4	4	Oil
Ascorbyl Palmitate	0.015	0.0075	phase
Lecithin	0.1	0.1	
Approved Color	0.01	0.01	
Approved Flavor	0.125	0.125	
Other additives	0.65	0.65	
Water	16	16	
Salt	2	1	Aqueous
Potassium Sorbate	0.1	0.1	phase

RBO = Rice bran oil

beaten using a stand mixer and whipping attachment (Moulinex, CMMF, China) at a whipping speed of ~260 rpm at 25 °C. The weight of the whipped margarine was measured after beating for 2, 5, 10, 15, 20, and 30 min and calculated as specific gravity at 25 °C by using the following equation (2).

$$\text{Specific gravity} = \frac{M3-M1}{M2-M1} \quad (2)$$

Where, M1= empty container weight (g), M2 = weight of the container filled with water (g), M3= container filled with whipped margarine (g).

Sensory evaluation of novel margarine

Sensory attributes such as taste, aroma, spreadability, and mouth feel were analyzed using a ranking test and done with 30 untrained panelists for each parameter separately for Novel and existing margarine and a competitor brand. Results were tabulated using the Friedman test at a 5% significant interval.

Statistical analysis

All the assays were performed in triplicate and statistical analysis was performed using Tukey's range test by Statistical Analysis System ver. 9.4 (SAS, Cary, USA).

RESULTS

Yield of rice bran oil

The oil yield of bran samples was 11.50±0.05% using solvent extraction with n-hexane as the solvent. However, since numerous cultivars with various properties were collected there may be an effect on the oil yield of rice cultivars.

Physicochemical parameters of RBO, RPO, PS, and RCNO

Measurement of physicochemical parameters of raw materials is a crucial step before blend preparation. Results obtained for color within the accepted limits were palm oil (3R) (SLS720, 2016), and coconut oil (2) (SLS32, 2017). For RBO, the results were within the limits (7.6- 13.6) by Krishna et al., 2006, where they referred 20 Lovibond units. RI results obtained in the study adhered to the current limits where, palm oil (1.4521-1.4541) (SLS720, 2016), coconut oil (1.4480-1.4492) (SLS32, 2017), RBO (1.4600-1.4730) (CXS 210-1999, 2019) and palm stearin (1.4482-1.4501) (SLS960, 2016) claiming that the oils fit for manufacturing. The relative density of raw materials was within the maximum allowable limits which were 0.8889-0.8896 g/ml (SLS720, 2016) for palm oil, 0.915-0.920 g/ml (SLS32, 2017) for coconut oil, 0.910-

0.929 g/ml (CXS 210-1999, 2019) for RBO and 0.8813- 0.8844 g/ml (SLS960, 2016) for palm stearin. SAP values of all the raw materials are within the limits which are 194-205 mg KOH (SLS720, 2016) for palm oil, 248-265 mg KOH

(SLS32, 2017) for coconut oil, 193-205 mg KOH (SLS960, 2016) for palm stearin, and 180-199 mg KOH (CXS 210-1999, 2019) for RBO. The physicochemical parameters of raw materials are mentioned below in Table 3.

Table 3. Physicochemical parameters of palm oil, coconut oil, rice bran oil, and palm stearin

Parameter	Palm oil	Coconut oil	Rice bran oil	Palm stearin
Color in 1/5.25 -inch Lovibond	45±0.00	2±0.00	10.3±0.00	45±0.01
Relative density (x° C/water at 20°C) (g/ml)	0.888±0.05	0.916±0.01	0.911±0.01	0.882±0.02
Refractive index nD (40/60°C)	1.453±0.01	1.448±0.01	1.465±0.02	1.448±0.01
Saponification value mgKOH/goil	197.2±0.88	248.3±0.5	180.7±0.5	195.5±0.5
FFA content (%)	0.07±0.03	0.072±0.04	0.22±0.01	0.11±0.01
PV (meq/kg)	2.4±0.33	1.9±0.3	3.7±0.2	1.9±0.01
SMP (°C)	35.5 ±0.4	33±0.5	20.72±0.1	50±0.6

FFA = Free fatty acids, PV = Peroxide value, SMP = Slip melting point

FFA of palm oil and coconut oil were within the SLS standards (0.1% max) (SLS720, 2016; (SLS32, 2017). Due to the high inherent FFA content in RBO (Punia et al., 2021), the maximum limit proposed by SLS was 0.3%. (Codex Alimentarius, 2019). PV of oils and fats give initial evidence of primary oxidation by the detection of peroxides formed where PV of 30- 40 meq/kg gives a noticeable rancid odor and a taste (Singh et al., 2022). PV of palm oil, coconut oil, and palm stearin was within the allowable maximum limits which were 3 meq/kg based on SLS specifications (SLS720, 2016; SLS32, 2017; SLS960, 2016). But, for RBO the proposed maximum limit by Codex Commission was 10 meq/kg and the obtained result was within the limit (Codex Alimentarius, 2019). SMP of oils and PS were within SLS standards where allowable minimum limits were 33-39 °C for PO, 25 °C for CNO, and 44 °C for PS. Therefore, the oils and palm stearin can be used to produce margarine.

Analysis of blends

Once the blends were prepared, their, FFA, PV, and SMP were again determined to verify that oil blends could be used to produce margarine. Even if there were significant differences ($P<0.05$) in FFA and PV among the novel blends and the SOB, all the values are within the limits adopted by SLS 277, 1987, which are FFA (0.15% max), PV (5 meq/kg) and SMP (28-34 °C). The obtained results are mentioned in Table 4.

The melting point provides a rough indication at which temperature the margarine gets smoother on the palate. The internationally accepted range of SMP for margarine is 28-34 °C (Nadeem *et al.*, 2018, p. 992). Even if SMP s of all the blends and SOB are significantly different ($P<0.05$) from each other, all the values were within the accepted range suggesting that margarine produced by all the above blends will instantly be melted in the mouth and tolerate the mechanical work done during the spreading. Since almost all the SMP values were less than human body temperature they could be used for preparation of table margarine with a proper mouth feel

Table 4. Chemical properties of novel and standard oil blends

Parameter	SOB	Blend 1	Blend 2	Blend 3	Blend 4
FFA (%)	0.064±0.001 ^c	0.06±0.002 ^d	0.067±0.00 ^c	0.071±0.001 ^b	0.075±0.001 ^a
PV (meq/kg)	1.56±0.020 ^e	1.63±0.01 ^d	1.68±0.01 ^c	1.72±0.01 ^b	1.82±0.01 ^a
SMP (°C)	34.4±0.10 ^a	33.8±0.6 ^b	33.2±0.1 ^c	31.8±0.06 ^d	29.6±0.6 ^e

SOB = Standard oil blend, FFA = Free fatty acids, SMP = Slip melting point, PO = Palm oil, Mean values with different superscripts within columns are significantly different. ($P < 0.05$)

Solid fat content

SFC is an indication of solid fat at specific temperatures. SFC at a specific temperature is a crucial predicament for the firmness and melting behavior of a certain fat blend (Abdolmaleki et al., 2022). The results are presented in Table 5. Higher the proportion of RBO showed a gradual decrease of SFC in all temperatures. That may be due to the replacement of coconut oil with RBO. Almost all the blends showed SFC values above 10% at 20 °C which is an indicator for resisting oiling off (Podchong et al., 2018). Almost all the samples exhibited less than 3% solid fat content (SFC) at temperatures above 33 °C, indicating

that none of the blends will have a waxy texture in the mouth (Abdolmaleki et al., 2020) (Podchong et al., 2018).

SFC value should be $\leq 32\%$ at ≤ 10 °C to be spreadable under refrigerator conditions (Abdolmaleki et al., 2020) (Podchong et al., 2018). Since none of the blends fulfill the above requirement, any above blend cannot be used to produce margarine that will be spreadable in refrigerated condition. However, since the SFC of all the blends is lower than 32% at 25 °C all the blends are suitable as hard stocks for margarines spreadable at room temperature. The SFC of novel RBO blends and SOB is shown as a function of temperature in Figure 1.

Table 5. The solid fat content of novel blends and SOB

Temperature (°C)	SOB	Blend 1	Blend 2	Blend 3	Blend 4
10	49.70 ±0.57 ^a	44.1±0.057 ^b	39.7 ±0.57 ^c	38.2±0.05 ^d	35.4 ±0.05 ^e
20	23.70 ±0.57 ^a	22.8±0.023 ^b	21.3±0.57 ^c	19.6± 0.02 ^d	19.2± 0.06 ^d
30	7.10±0.57 ^a	6.92±0.005 ^b	6.8±0.57 ^c	6.0±0.05 ^d	5.2± 0.05 ^e
40	0.27±0.57 ^{ab}	0.28±0.005 ^a	0.22±0.57 ^{ab}	0.26±0.005 ^{ab}	0.2±0.005 ^b

SOB = Standard Oil Blend, Mean values with different superscripts within columns are significantly different. ($P < 0.05$)

Fatty acid profile of oil blends

Fatty acid compositions show the modification of fatty acids in terms of saturation and unsaturation. The amount of each fatty acid present in all novel blends was significantly different ($P < 0.05$) from the SOB. Therefore, there was a significant modification of the fatty

acid profile in novel oil blends by the addition of RBO. Blend 4 (20% RBO) showed significant improvement in C18:1(Oleic acid) C18:2 (Linoleic acid) and C18:3 (α Linolenic acid) among all other blends including the SOB. The increment in linoleic acid and α -linolenic acid in blend 4 will be nutritionally favorable since they are essential fatty acids. Further, blend 4 had the least amount of C12:0

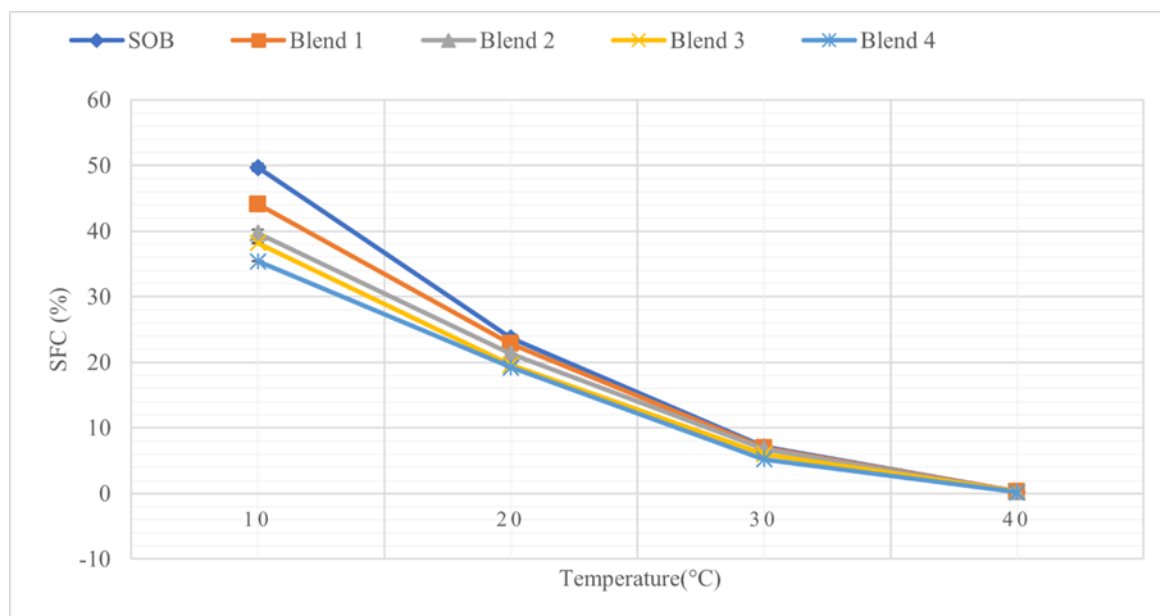


Figure 1. The change of SFC at varying temperatures of novel and Standard oil blends.

and C14:0 fatty acid amounts in blend 4 compared to other blends. The fatty acid profile of novel oil blends and SOB is presented in Table 6.

There were significant differences ($P < 0.05$) in total SAF%, PUFA%, and MUFA% among all the blends including SOB. Blend 4 showed the least SAF% whereas SOB showed the highest SAF%. Thus, in blend 4, SAF% has significantly decreased to $42.61 \pm 0.06\%$ from 52.88 ± 0.16 . The highest MUFA and PUFA content was found in also in blend 4 whereas

the least was found in SOB. Therefore, there was a significant improvement in Blend 4 in PUFA ($10.34 \pm 0.01\%$) and MUFA ($22.4 \pm 0.02\%$). Since WHO recommends replacing SAF as much as possible with PUFA and MUFA, all novel blends were more nutritionally beneficial than SOB. Hence, considering the above desirable features, blend 4 was the optimum ratio to develop novel margarine which will be an ideal option for health-conscious individuals than the existing margarine. The calculated results are mentioned in Table 7.

Table 6. The fatty acid composition (%) of novel oil blends and SOB

Fatty acid	C12	C14	C16	C18	C18:1	C18:2	C18:3
SOB	21.18 ± 0.01^a	8.68 ± 0.03^a	20.12 ± 0.06^e	2.90 ± 0.1^a	15.58 ± 0.05^e	4.1 ± 0.04^e	0.14 ± 0.00^e
Blend 1	18.81 ± 0.01^b	7.77 ± 0.05^b	20.82 ± 0.02^d	2.69 ± 0.07^b	17.43 ± 0.03^d	5.52 ± 0.01^d	0.17 ± 0.00^b
Blend 2	16.42 ± 0.11^c	6.89 ± 0.03^c	21.56 ± 0.04^c	2.65 ± 0.01^b	19.12 ± 0.09^c	7.12 ± 0.03^c	0.19 ± 0.00^c
Blend 3	14.1 ± 0.05^d	5.87 ± 0.04^d	22.43 ± 0.05^b	2.64 ± 0.01^b	21.02 ± 0.06^b	8.69 ± 0.06^b	0.22 ± 0.00^d
Blend 4	11.6 ± 0.021^e	5.1 ± 0.001^e	23.28 ± 0.03^a	2.63 ± 0.01^b	22.84 ± 0.03^a	10.1 ± 0.01^a	0.24 ± 0.00^a

SOB = Standard oil blend, Mean values with different superscripts within columns are significantly different ($P < 0.05$)

Table 7. Comparison of total SAF and unsaturated fat content (%) of novel blends and SOB

Fatty acid	SAF	MUFA	PUFA
SOB	52.88±0.16 ^a	15.58±0.04 ^e	4.23±0.04 ^e
Blend 1	50.10±0.08 ^b	17.43±0.03 ^d	5.69±0.01 ^d
Blend 2	47.50±0.04 ^c	19.12±0.09 ^c	7.31±0.03 ^c
Blend 3	45.04±0.08 ^d	21.03±0.06 ^b	8.91±0.05 ^b
Blend 4	42.61±0.06 ^e	22.40±0.02 ^a	10.34±0.01 ^a

SOB = Standard Oil Blend, Mean values with different superscripts within columns are significantly different. ($P < 0.05$)

Quantification of γ -oryzanol

γ -oryzanols in rice are well known for their antioxidant activity due to the components such as ferulic acid esters present in it (Andriani et al., 2022). The number of γ -oryzanols varies according to the rice cultivar, extraction method, storage time, and method of value addition. According to the results presented in Table 8, there is no significant difference ($P > 0.05$) in γ -oryzanol concentration among dewaxed, refined, and novel RBO

blends whereas the highest amount was found in crude RBO which means there was no significant loss in the degumming, dewaxing, and refining processes. Oryzanol content ranged from 10-17 mg/ g when physically refined, but 1-2 mg/ g when chemically refined (Punia et al., 2021). But the number of γ -oryzanols in this study was less in crude RBO (6.85 mg/g $\pm$ 0.08) when compared to different studies (9.8 mg/ g) and (10 mg/g) (Godber and Xu, 2021; Sahini and Mutegoa, 2023).

Table 8. Content of γ -oryzanols found in RBO and RBO blends

Type	γ -oryzanols content (mg/g)
Crude RBO	6.85 $\pm$ 0.008 ^a
Dewaxed RBO	4.47 $\pm$ 0.02 ^{ab}
Refined RBO	3.94 $\pm$ 0.002 ^{ab}
Blend 1	0.88 $\pm$ 0.1 ^b
Blend 2	1.02 $\pm$ 0.002 ^{ab}
Blend 3	2.25 $\pm$ 0.3 ^{ab}
Blend 4	3.56 $\pm$ 0.5 ^{ab}

RBO = Rice bran oil, Mean values with different superscripts within columns are significantly different ($P < 0.05$)

However, the γ -oryzanols content of red-grained rice varieties ranges from 2.983 to 5.286 mg/g in bran of brown rice, when extracted with acetone, which the results obtained in this study are also within the range (Gunaratne et al., 2012), lesser oil yield, time-lapse occurred from polishing to refining and the type and season of the cultivar may be the possible reasons for lesser γ -oryzanols content in this study.

Oxidative stability of oils

The oxidative stability gives an indication of the shelf-life based on the formation of secondary oxidative products, in terms of mean induction time. The higher the induction time, the higher the oxidative stability and shelf-life. Table 9 shows the results of an accelerated study done for blends in terms of induction time at 140 °C.

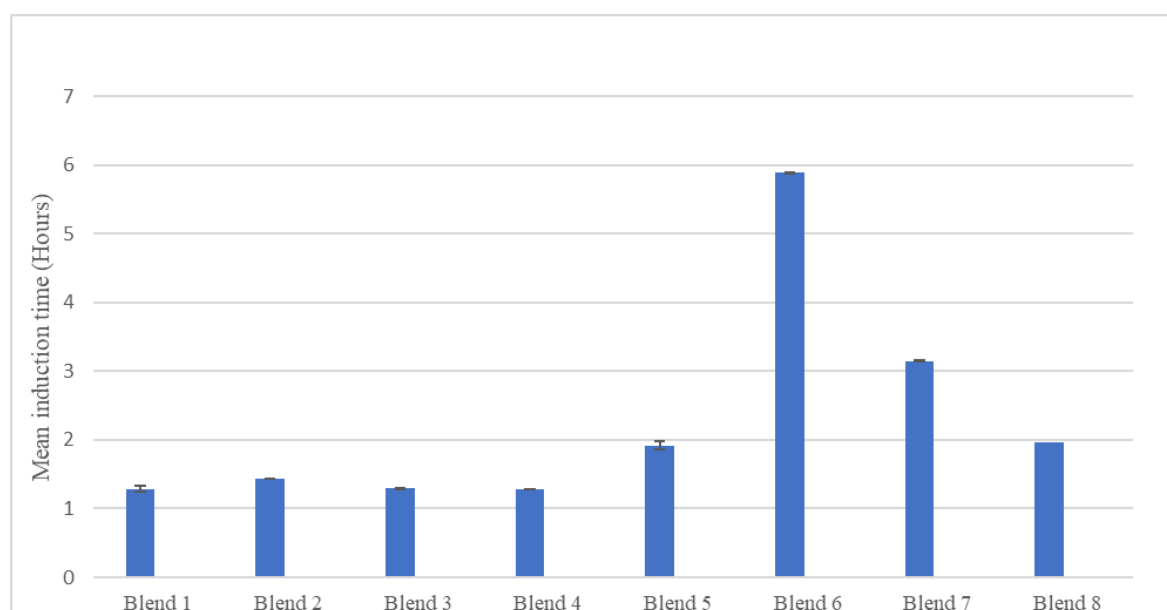
Table 9. The mean induction time and oxidative stability index of oil blends at 140 °C

Blend	Mean Induction time (hours)	Oxidative stability index at 25 °C (years)
1	1.285 ± 0.01 ^e	0.65
2	1.427 ± 0.06 ^d	0.76
3	1.290 ± 0.00 ^e	0.66
4	1.279 ± 0.01 ^e	0.64
5	1.920 ± 0.00 ^c	1.16
6	5.89 ± 0.06 ^a	1.73
7	3.15 ± 0.010 ^b	1.73
8	1.967 ± 0.06 ^c	1.18

Mean values with different superscripts within columns are significantly different ($P < 0.05$)

The highest oxidative stability (highest induction time) was shown by blend 6 (blend with TBHQ). There was no significant difference ($P > 0.05$) in induction times among blends of 5%, 15%, and 20% RBO whereas the 10 % RBO added blend showed the lowest induction time. Blend 7 (Rosemary extract added blend) showed the second-highest

induction time with the second-highest oxidative stability. AP added blend (blend 5) showed the third highest oxidative stability, even higher than the RBO blends. There was no significant difference ($P > 0.05$) in oxidative stability between blend 5 and blend 8 (20% RBO+0.0075% AP and 0.015% AP). The results are presented in Figure 2.

**Figure 2.** The change of mean induction of different oil blends at 140 °C

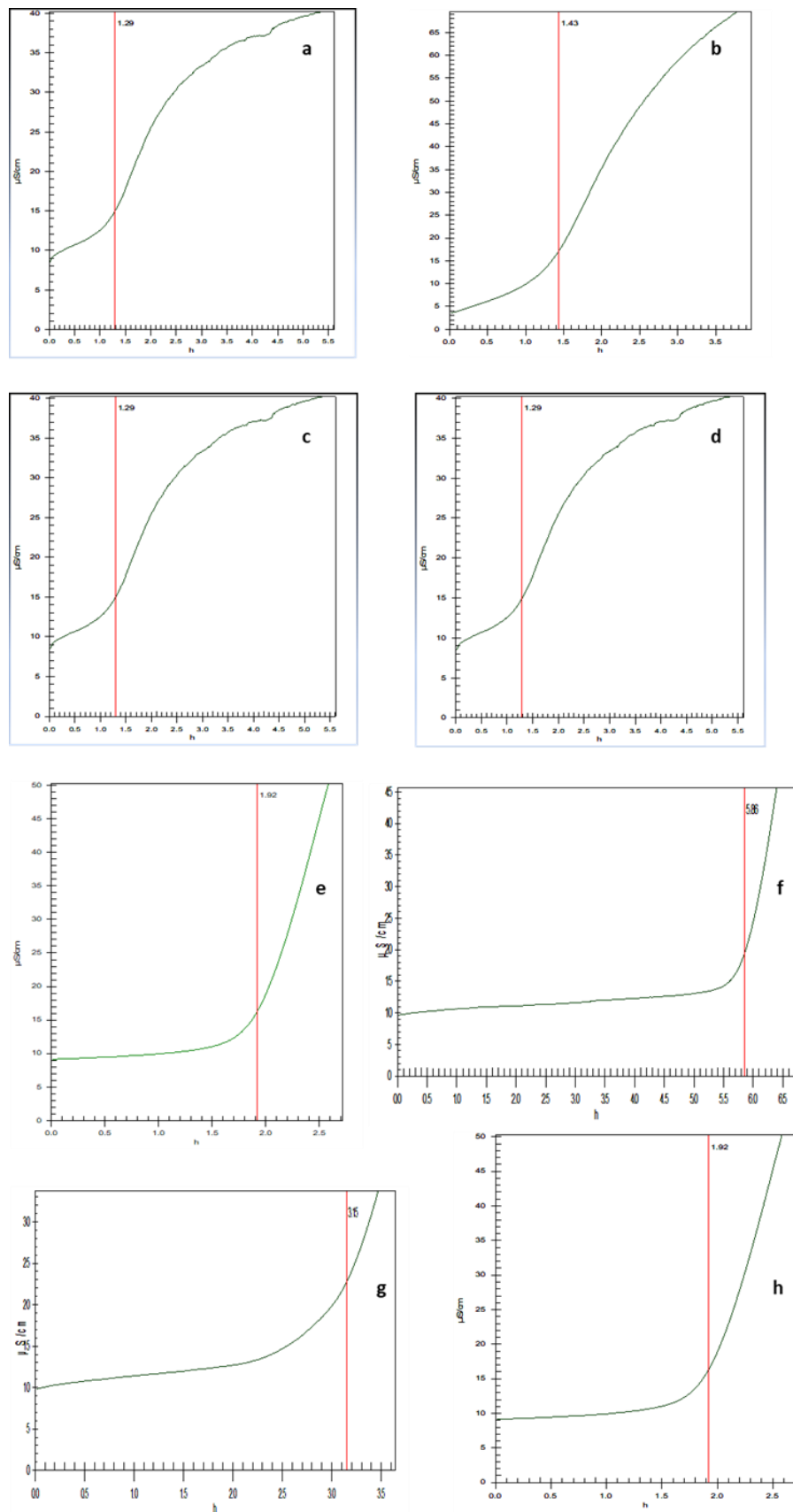


Figure 3. Electrical conductivity against time :(a-h) Electrical conductivity against time for blends 1-8 at 140 °C consecutively

Even if RBO can be used as a natural antioxidant, the RBO used in the study had no significant effect compared to synthetic antioxidants. Since the original γ -oryzanol content of this study was less than the previous studies, antioxidant efficacy may have been lesser compared to synthetic antioxidants. Therefore, to obtain the maximum antioxidant efficacy of RBO, apart from adding raw RBO, extracted and purified oryzanol can be added (Krishna et al., 2015). But, since antioxidants work in synergy among themselves, not only oryzanol but also other antioxidants such as tocopherols, squalene etc. must be quantified (Liu et al., 2023). On the other hand, during collection, since bran belongs to numerous cultivars was getting mixed, and the time taken for stabilization after polishing may have deviated from the quality of RBO which is practically very difficult to control. However, since blend 8 has no significant difference ($P>0.05$) in oxidative stability from blend 5, this kind of raw RBO can be added as an antioxidant mixture to replace synthetic

antioxidants. Hence, among other blends, blend 8 (RBO 20 % and 0.0075%) had a possibility of incorporation into novel margarine. Figure 2 shows the mean induction time against the blends at 140 °C.

Margarine preparation

Considering all the parameters tested above, blend 8 was the optimum blend for novel margarine preparation.

Whipping properties

Good whipping properties are important for air entrapment during making batters for bakery/commercial applications (Podchong et al., 2018). Air is incorporated and entrapped in the fat phase during whipping or aeration, which will enhance the volume and smooth texture of the final product. Better whipping properties are associated with lower specific gravity (Podchong et al., 2018). The whipping properties of novel and existing margarine are shown in Figure 4.

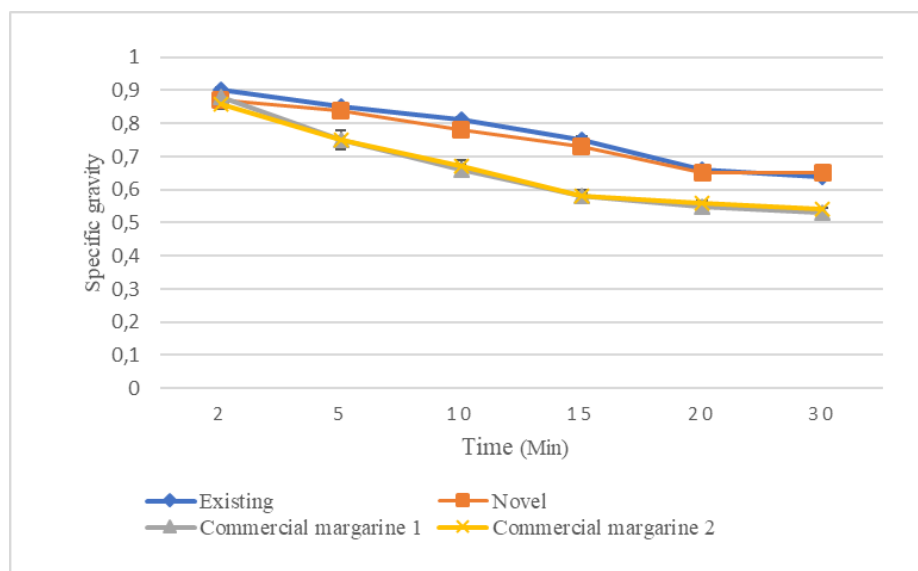


Figure 4. The specific gravity of margarine at 25 °C as a function of whipping time

The specific gravity of all types of margarine at 25 °C was decreased over time, proving that more air was incorporated with whipping/ beating, ultimately resulting in an increment in

volume. But both novel and existing margarine showed a smaller drop in specific gravity and similar patterns with time when compared to commercial margarine. That implied novel and

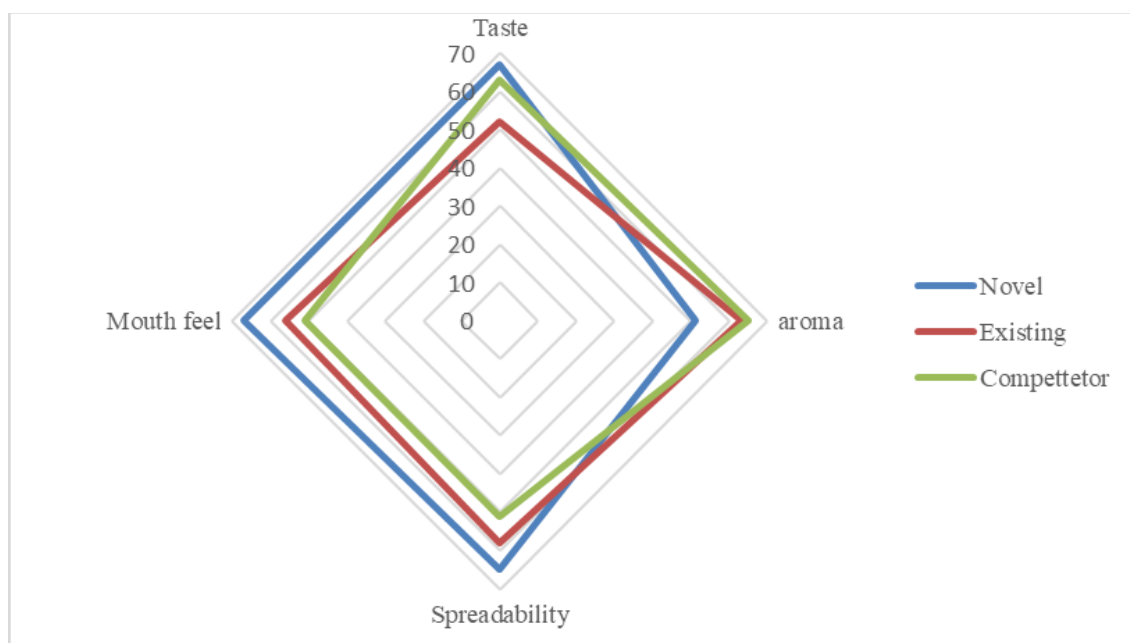


Figure 5. Sensory analysis of taste, aroma, mouth feel, and spreadability for both novel, existing margarine, and competitor margarine

existing margarine had poor whipping properties. Therefore, they cannot be used for commercial bakery applications.

Sensory analysis

There was no significant difference ($P > 0.05$) among novel, existing margarine and a competitor brand based on consumer preference for all the sensory attributes taken, i.e., taste, aroma, mouth feel, and spreadability. The results are presented in Figure 5.

DISCUSSION

The γ -oryzanol content of commercially and locally available rice bran samples which have been collected was comparatively less than that of results of previous studies. That may be due to the poor processing and storage practices after harvesting. Therefore, even if there is a significant amount of γ -oryzanol found in the specific RBO, it cannot be taken as a single antioxidant to replace current synthetic antioxidants, which can be added with antioxidant mixtures. Hence, showing oxidative stability with no significant

difference ($P > 0.05$) with AP was concluded that 20% RBO with 0.0075% AP can be taken to produce the novel margarine. Novel margarine which could only be spreadable at room temperature, resisted oiling off and did not demonstrate waxiness in the mouth. With the incorporation of RBO, the nutritionally favorable fatty acid profile was modified with a significant reduction in total SAF and increments in PUFA and MUFA with a significant increment in the essential fatty acids. There was no significant difference ($P > 0.05$) found on consumer preference for both novel margarine, and existing margarine. But since novel margarine cannot be used for commercial bakery applications, it can be used as a table margarine and for household purposes. However, due to the presence of γ -oryzanol and other nutritional modifications the novel margarine can be categorized as a functional food. But, to get the maximum effect on γ -oryzanol, either it needs to be purified or extracted and added to the product and specific processing steps need to be modified. Moreover, considering the synergistic effect of antioxidants found in RBO, quantification, and refining should be modified to preserve all of

them. When popularizing RBO among the public, a proper supply chain of rice bran to the oil extractors should be provided and well monitored.

CONCLUSION

Locally and commercially available rice bran samples can be effectively utilized to extract rice bran oil and produce functional margarine by reducing the saturated fat content. Further, it can be used to reduce synthetic antioxidant usage. But to use it as a natural antioxidant separately, for fat-based food applications, a much more modified, streamlined, and synergized extraction and quantification mechanism should be adopted.

AUTHOR CONTRIBUTIONS

Conceptualization, T.M., and O.C.P.D.S.; methodology, A.A.E.; software, A.A.E.; validation, T.M., O.C.P.D.S., and S.S.; formal analysis, A.A.E.; investigation, A.A.E.; resources, A.A.E.; data curation, A.A.E.; writing - A.A.E.; draft preparation, A.A.E.; writing - review and editing, T.M.; visualization, T. M.; supervision, O.C.P.D.S.; project administration, T. M.; funding acquisition, O.C.P.D.S. All authors have read and agreed to the published version of the manuscript.

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DATA AVAILABILITY STATEMENT

Available upon request.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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