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FATTY ACID COMPOSITION, OXIDATIVE STABILITY, AND RADICAL SCAVENGING ACTIVITY OF RICE BRAN OIL BLENDS

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ABSTRACT

Rice bran oil is less expensive non-conventional oil which has been found to improve the stability of the blended oil due its nutrient composition. The purpose of present study was to develop a healthier and stable blend using rice bran oil and other conventional oils. Therefore, six rice bran oil blends were prepared in two ratios i.e. 80:20 and 70:30. These rice bran oil blends were analysed for fatty acid composition, oxidative stability, and antioxidant activity. Consequently, rice bran oil + olive oil and rice bran oil + soybean oil contained highest amount of monounsaturated fatty acids (47.6%) and polyunsaturated fatty acids (61.6%) respectively in the ratio of 70:30. Saturated fatty acids (SFA) were found be highest (24.5%) in rice bran oil + palm oil (70:30). Rice bran oil + palm oil (70:30) and rice bran oil + olive oil (70:30) showed least percent increase in peroxide formation i.e. 21.4 and 30.3 percent respectively. Rice bran oil + sunflower oil (70:30) and rice bran oil + olive oil (70:30) had highest content of alpha tocopherol equivalent i.e. 295.1 and 249.5mg/Kg respectively. Oryzanol content was found to be highest in rice bran oil + olive oil and rice bran oil + soybean oil i.e. 2294.0 mg/Kg (80:20) whereas the highest value of total natural antioxidants (2568.7 mg/kg) was observed in rice bran oil + sunflower oil (70:30). Rice bran oil + olive oil (70:30) showed highest (67.7%) radical scavenging activity towards 2, 2-diphenyl-1-picrylhydrazyl free radicals.

Keywords: Fatty acid composition, natural antioxidants, oxidative stability, radical scavenging activity, rice bran oil.

INTRODUCTION

Rice Bran Oil (RBO) is miracle product obtained from the outer brown layer of rice. Current annual world rice bran oil production is estimated to be less than 8 lakh tons or about 1 per cent of all vegetable oils used for human consumption (Kusum *et al.,* 2011). India is the second largest producer of crude rice bran oil in the world. India produces 120 million tons of paddy annually which contain 5-6 per cent of rice bran, leading to production of 6-7 million tons of rice bran oil. Rice bran contains 16 per cent oil, hence there is a potential for production of 1- 1.2 million tons of rice bran oil in the country but the actual production of rice bran oil is only 0.5-0.6 million tons (Usha and Premi, 2011).

RBO is an excellent cooking medium because it is nutritionally superior, contains more micronutrients, longer shelf life, more stable at higher temperature, gives better taste & flavour to food items, frying takes less time so saves energy and economical due to 15 per cent less absorption of oil during frying (Sharma, 2002). RBO has cholesterol-lowering properties due to the presence of a component called oryzanol (Visser et al., 2000). Oryzanol is also beneficial in a range of other ailments including gastro-intestinal disorders and nerve imbalance. It is also known to have a significant effect on menopause by alleviating the menopausal symptoms like hot flashes (Patel and Naik, 2004). It is used as an ergogenic supplement by body builders and athletes (Fry et al., 1997). Rice bran oil's other components like tocotrienols and squalene have powerful anti-cancer and anti-ageing properties (Sierra et al., 2005). Taking advantage of the valuable fact that micronutrient levels are so adequate in rice bran oil, it was considered that value of any other edible oil could be remarkably increased by addition of even small amounts of rice bran oil (Adhikari, 2002).

A wide range of vegetable oils are available in the market. However, some vegetable oils are not up to standards to meet consumer satisfaction in terms of their physicochemical properties and stability (Reyes-Hernandez et al., 2007). Blending of vegetable oils is gaining popularity due to its advantages like improved thermal stability, oxidative stability, and nutritional benefits (Nor Aini and Sabariah, 2005; Nor Hayati et al., 2002). Blending of oil with rice bran oil has been found to improve the stability of the blend during frying and storage (Gopal et al., 2005). In addition, RBO is less expensive non-conventional oil and blending it with other expensive conventional vegetable oils will produce lower cost rice bran oil blends.

Hence, the purpose of this study was to develop healthier and stable blends of rice bran oil and also to study the fatty acid composition, oxidative stability, and antioxidant activity of these blends having rice bran oil as the major component.

MATERIALS AND METHODS

MATERIALS

Refined rice bran oil (RBO) and other refined vegetable oils viz., olive oil (OO), groundnut oil (GO), soybean oil (SOO), sunflower oil (SO), mustard oil (MO) and plam-olein oil (PO) were purchased from local market. All the analytical and GC grade chemicals and solvents used were supplied by Himedia (Mumbai, India).

METHODS

PREPARATION OF BLENDS

A 100 ml mixture of RBO and other vegetable oil were placed in duplicate in 250-ml beakers for each blend and were mixed by using a mechanical stirrer at 180 rpm for 15 min. Blends of rice bran oil viz. RBO+OO, RBO+GO, RBO+SOO, RBO+SO, RBO+MO and RBO+PO were prepared in two ratios i.e., 80:20 and 70:30 (Bhatnagar et al., 2009). These blends were analysed for fatty acid composition, oxidative stability, natural antioxidants and radical scavenging activity.

The basis of selection of six blends was a combination of three factors: (1) commonly consumed vegetable oils (2) presence of adequate amounts of monounsaturated fatty acid (MUFA), polyunsaturated fatty acid (PUFA), and natural antioxidants (3) lower cost of the blend when compared to individual oil. The selection of two ratios i.e. 80:20 and 70:30 was based on recommendation given by Prevention of Food Adulteration Act (PFA) 1954. According to PFA 4th Amendment Rules 1992, blending of any two vegetable oils (wherein the component oil used in the admixture is not less than 20%) has been permitted.

FATTY ACID COMPOSITION BY GAS CHROMATOGRAPHY (GC)

Oil samples were analysed for their fatty acid composition by gas chromatography using fatty acid methyl esters (FAME) preparation (Appleqvist, 1968). FAMEs were analysed on a gas chromatograph (Varian CP 3800, USA), equipped with a flame ionization detector (FID) and a fused silica capillary column (50 m x 0.25 mm i.d.), coated with CP-SIL 88 as the stationary phase. The oven temperature was programmed at 200 °C for 13 min. The injector and FID were at 250 °C. A reference standard FAME mix (Supelco Inc.) was analyzed under the same operating conditions to determine the peak identity. The FAMEs were expressed as relative area percentage.

OXIDATIVE STABILITY

Each blend was placed in beakers (50- ml) capacity and incubated at 37°C and 55 % RH in a lab incubator to study the oxidative stability of the blends over a period of 4 weeks (28 days). Samples were withdrawn at weekly intervals and analysed for their peroxide value (PV). The PV is a titration measure of all peroxides and lipid oxidation products that will oxidize the potassium iodide under operating conditions. Five grams of the oil sample was poured into a 250 ml flask. Thirty millilitres of glacial acetic acid/chloroform (3:2, v/v) solutions were added and stirred. A stopper was inserted and the flask was shaken for 1 min and left for 5 min in the dark at 15–25 ^{o}C. Thirty millilitres of distilled water was added, and the librated iodine was titrated with 0.01 N $Na_2S_2O_3$, using starch as indicator. The PV was calculated following the AOCS (2003) method.

ANTIOXIDANT ACTIVITY

To analyze antioxidant activity of blends, natural antioxidants (oryzanol, α-tocopherol equivalent) and radical scavenging activity (RSA) towards DPPH radicals were determined.

NATURAL ANTIOXIDANTS

The alpha tocopherol equivalent was determined by Emmerie Engel assay modified by Baker and Frank (1988). Three stoppered centrifuge tubes were taken and labelled as standard, sample, and blank. To these labelled tubes, 0.5 ml of DL-*α*-Tocopherol acetate (standard), 0.5 ml of blended oil *(*sample*)* and 0.5 ml of distilled water *(*blank*)* were added respectively. In each centrifuge tube, 0.5ml of ethanol and 0.5ml of xylene were added. All the three stoppered centrifuge tubes were mixed and centrifuged for 15min. In other three clean stoppered tubes, 0.5ml of each xylene layer was transferred. To this 0.5ml of dipyridyl reagent was added and 0.5 ml of this mixture was pipetted into spectrophotometer cuvettes (Systronics UV-VIS-108, Bangalore, India) and the absorbance of sample and standard against the blank was read at 460 nm. To the blank, standard and sample, 0.33 ml of ferric chloride reagent was added and mixed for 30 seconds. After 1.5 minutes of the addition, zero setting was done at 520 nm and absorbance of the sample and standard against the blank was read. The alpha tocopherol equivalent was calculated by using this formula:
Alpha tocopherol equivalent (mg%) = [alpha tocopherol in standard (mg %) x {sample OD_{520} – (0.29 x sample OD_{460})}/standard OD_{520}]

Oryzanol content of blended oil was determined by a spectrophotometric method (Gopal et al., 2006) 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 (Systronics UV-VIS-108 spectrophotometer, Bangalore, India). The oryzanol content was calculated by using the formula:

[(A/W) X (100/358.9)]

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

RADICAL SCAVENGING ACTIVITY (RSA) TOWARD DPPH RADICALS

DPPH radical scavenging activity was measured using the method described by Erasto et al. (2007) and Miraliakbari and Shahidi (2008). This assay is based on the determination of the concentration of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) methanolic solution, after adding the antioxidants. DPPH concentration is reduced by the existence of an antioxidant at 515 nm and the absorption gradually disappears with time. A 0.1 mM methanolic solution of DPPH was prepared. The oil samples (1 ml after tenfold dilution) were placed in test tubes and a 2-ml aliquot of DPPH methanolic solution was added and the mixture was vortexed for 20 s at ambient temperature. Against a blank of pure methanol without DPPH, the decrease in absorption at 515 nm was measured in 1-cm quartz cells after 1, 30, and 60 min of mixing, using a spectrophotometer (Systronics UV-VIS-108, Bangalore, India). RSA toward DPPH radicals was estimated from the differences in absorbance of methanolic DPPH solution with or without sample (control) and the inhibition percent was calculated using the following equation:

% inhibition = [(absorbance of control - absorbance of test sample)/absorbance of control] X 100.

STATISTICAL ANALYSIS

All the determinations were carried out in triplicate and the results are expressed as mean ± standard error. One way analysis of variance (ANOVA), factorial completely randomized design (CRD), factorial randomized block designs (RBD) and their statistical significance ($p \leq 0.05$) was ascertained using a computer programme package (CPCS1). Pearson correlation analysis and statistical significance ($p \leq 0.01$) was also carried out to determine the relationship between natural antioxidants and DPPH radical scavenging activity.

RESULTS AND DISCUSSION

FATTY ACID COMPOSITION

The fatty acid composition of the blends of rice bran oil is given in Table 1.

RBO+OO (70:30) and RBO+GO (80:20) contained highest amount of MUFA (oleic acid) i.e. 47.6 and 36.9 per cent respectively. Several studies reported the beneficial effect of MUFA on cardiovascular and diabetic risk factors (Schwingshackl and Hoffmann, 2012). Oleic acid had been described to reduce the cardiovascular risk by reducing blood lipids, mainly cholesterol (Coni et al., 2000; Turner et al., 2005; Lopez-Huertas, 2010; Stephens et al., 2010). RBO+SOO contained highest percentage of PUFA i.e. 61.6 followed by RBO+MO (59.2 %) and RBO+SO (58.5 %) in the ratio 70:30. Similar trend was also observed in the ratio of 80:20 for PUFA content in RBO+SOO (52.6%), RBO+MO (50.8%) and RBO+SO (49.7%). Scientific studies demonstrated the potential beneficial effects of PUFA for chronic diseases including cancer, insulin resistance and cardiovascular disease (Ruxton et al., 2004; Gibson et al., 2011; Anderson and David, 2009). Saturated fatty acids (SFA) were found be highest in RBO+PO i.e. 24.5 per cent in the ratio of 70:30. Similar findings were also reported by Fan et al. (2012). Significant ($p \leq 0.05$) difference was found in fatty acid composition of rice bran oil blends.

OXIDATIVE STABILITY

Oxidative stability of oil can be improved by modification of fatty acid composition and addition of antioxidants to the oil (Moussata and Akoh, 1998). The oxidative stability of rice bran oil blends is given in Table 2. Significant ($p \leq 0.05$) difference was found in peroxide value of all rice bran oil blends after 28 days. RBO+PO showed least percent increase in peroxide formation i.e. 24.9 and 21.4 percent in the ratio of 80:20 and 70:30 respectively after the storage period of 28 days. It could be due to the presence of SFA and MUFA in this blend. The findings of the study were in line with previous studies (Adhikari and Hu, 2012). The percentage of peroxide formation in RBO+OO was 36.3 and 30.3 in the ratio of 80:20 and 70:30 respectively after 28 days. The percent increase in peroxide formation in RBO+GO and RBO+MO was found to be almost same i.e. 39.0 and 39.3 in the ratio of 80:20 and 36.4 and 33.9 in the ratio of 70:30 respectively. The low peroxide formation in RBO+OO and RBO+GO could be due to presence of high amount of MUFA (oleic acid) as shown in Table 1. Vegetable oils with high amounts of oleic acid are slower in developing oxidative rancidity during shelf life or oxidative decomposition during frying than those oils that contain high amounts of polyunsaturated fatty acids (Abdulkarim and Ghazali, 2012). RBO+SOO and RBO+SO showed highest percent increase in peroxide formation i.e. 55.2 and 46.7 per cent in the ratio of 80:20 and 50.8 and 45.0 per cent in the ratio of 70:30 respectively. These blends contained highest amount of PUFA (Table 1). Recent studies reported that oxidative stability was inversely proportional to PUFA content of vegetable oil (Bhatnagar et al., 2009).

ANTIOXIDANT ACTIVITY

NATURAL ANTIOXIDANTS

The term "total natural antioxidants" collectively refers to total alpha tocopherol equivalent and oryzanol content in all blended oils. Tocopherols belong to a class of phenolic antioxidants which can inhibit lipid autoxidation by scavenging free radicals and by reacting with singlet oxygen. In vegetable oils alpha-tocopherol inhibits the effects of singlet oxygen during sensitized photoxidation (Min and Boff, 2002). Figure 1 illustrates that RBO+SO in the ratio of 80:20 and 70:30 had highest content of alpha tocopherol equivalent i.e. 247.3 and 295.1 mg/Kg followed by RBO+OO in which content of alpha tocopherol equivalent

Table 1 Fatty acid composition of blended oils

Ratio 80:20								CD•
Fatty acid (%)	**RBO**	**RBO+OO**	**RBO+GO**	**RBO+SOO**	**RBO+SO**	**RBO+MO**	**RBO+PO**	
Palmitic acid (C16:0)	14.5±0.8	20.6±0.4	16.8±0.3	16.7±0.8	20.5±0.7	16.4±0.6	21.8±1.0	1.64
Stearic acid (C18:0)	0.9±0.3	0.5±0.1	0.2±0.1	0.3±0.1	0.4±0.1	0.5±0.2	0.7±0.2	NS
Oleic acid (C18:1)	38.0±1.3	42±0.9	36.9±1.0	30.4±0.6	29.4±0.7	32.3±0.6	33.4±0.5	1.44
Linoleic acid (C18:2)	46.6±1.0	36.9±0.7	46.1±0.9	51.7±1.9	49.7±0.4	39.1±1.2	44.1±1.2	2.52
Linolenic acid (C18:3)	ND	ND	ND	0.9±0.4	ND	8.6±0.4	ND	1.74
Arachidic acid (C20:0)	ND	ND	ND	ND	ND	3.1±0.3	ND	0.70
SFA %	15.4±1.0	21.1±0.3	17±0.3	17±0.9	20.9±0.6	16.9±0.4	22.5±0.8	1.49
MUFA %	38.0±1.3	42±0.9	36.9±1.0	30.4±0.6	29.4±0.7	32.3±0.6	33.4±0.5	1.44
PUFA %	46.6±1.0	36.9±0.7	46.1±0.9	52.6±1.5	49.7±0.4	50.8±1.1	44.1±1.2	2.02
Ratio 70:30								
Fatty acid (%)	RBO	RBO+OO	RBO+GO	RBO+SOO	RBO+SO	RBO+MO	RBO+PO	CD•
Palmitic acid (C16:0)	14.5±0.8	18.7±1.4	14.8±1.3	14.4±0.6	13.8±0.6	14.9±0.9	23.9±0.6	2.28
Stearic acid (C18:0)	0.9±0.3	0.3±0.2	0.1±0.0	0.5±0.2	0.6±0.2	0.3±0.1	0.6±0.2	NS
Oleic acid (C18:1)	38.0±1.3	47.6±1.4	30.7±0.4	23.5±0.6	27.1±0.2	25.6±0.8	30.3±2.1	2.34
Linoleic acid (C18:2)	46.6±1.0	33.4±1.1	54.4±1.1	60.5±0.6	58.5±0.9	45.2±0.3	45.2±1.2	1.71
Linolenic acid (C18:3)	ND	ND	ND	1.1±0.2	ND	9.8±0.9	ND	1.39
Arachidic acid (C20:0)	ND	ND	ND	ND	ND	4.2±0.9	ND	1.83
SFA %	15.4±1.0	19±1.6	14.9±1.3	14.9±0.4	14.4±0.8	15.2±0.8	24.5±0.4	2.33
MUFA %	38.0±1.3	47.6±1.4	30.7±0.4	23.5±0.6	27.1±0.2	25.6±0.8	30.3±2.1	2.34
PUFA %	46.6±1.0	33.4±1.1	54.4±1.1	61.6±0.4	58.5±0.9	59.2±0.3	45.2±1.2	1.68

Values are expressed as mean ±SE,• = Significant 5% , NS-Non significant
RBO rice bran oil, OO olive oil, GO groundnut oil, SOO soybean oil, SO sunflower oil, MO mustard oil, PO palm oil, ND- Not detected,
SFA saturated fatty acids, MUFA monounsaturated fatty acids, PUFA polyunsaturated fatty acids

Table 2 Peroxide values (meq/Kg) of blended oils for oxidative stability after weekly interval

	Peroxide value						Percent increase in peroxide value			
Blend	**0**	**7 days**	**14 days**	**21 days**	**28 days**	**CD***	**7 days**	**14 days**	**21 days**	**28 days**
RBO (100%)	0.62±0.07	0.72±0.07	0.98±0.10	1.55±1.85	3.24±0.22		13.9	26.5	36.8	52.2
Ratio 80:20										
RBO+OO	0.33±0.03	0.36±0.02	0.47±0.42	0.65±0.51	1.02±0.33	0.15	7.4	23.4	27.7	36.3
RBO+GO	1.13±0.06	1.27±0.07	1.69±0.08	2.46±0.08	4.03±0.37		10.8	24.9	31.3	39.0
RBO+SOO	0.73±0.02	0.96±0.12	1.45±0.09	2.29±0.09	5.11±0.47		23.6	33.8	36.7	55.2
RBO+SO	1.38±0.10	1.45±0.04	2.01±0.08	3.27±0.14	6.13±0.31		16.3	28.0	38.4	46.7
RBO+MO	1.33±0.12	1.52±0.07	2.09±0.26	3.09±0.26	5.09±0.47		12.3	27.3	32.4	39.3
RBO+PO	1.10±0.01	1.27±0.03	1.45±0.17	1.69±0.17	2.25±0.33		10.8	12.4	14.2	24.9
CD*	0.17									
Ratio 70:30										
RBO+OO	0.53±0.04	0.57±0.03	0.71±0.48	0.92±0.04	1.32±0.44	0.18	5.9	20.2	22.8	30.3
RBO+GO	1.53±0.07	1.67±0.05	2.15±0.06	3.02±0.07	4.75±0.33		8.2	22.3	28.8	36.4
RBO+SOO	0.93±0.06	1.19±0.18	1.70±0.09	2.58±0.06	5.24±0.44		21.6	30.0	34.1	50.8
RBO+SO	1.40±0.00	1.60±0.00	2.20±0.06	3.35±0.00	6.09±0.40		12.5	27.3	34.3	45.0
RBO+MO	1.73±0.09	1.93±0.07	2.53±0.28	3.59±0.09	5.43±0.33		10.3	23.7	29.4	33.9
RBO+PO	1.47±0.02	1.61±0.06	1.82±0.07	2.09±0.02	2.66±0.47		8.9	11.5	12.9	21.4
CD*	0.24									

Values are expressed as mean ±SE, • = Significant 5%
RBO rice bran oil, OO olive oil, GO groundnut oil, SOO soybean oil, SO sunflower oil, MO mustard oil, PO palm oil

Table 3 Correlation between total natural antioxidants and DPPH Radical scavenging Activity of blended oils

Blend	Oryzanol (mg/kg) [a]	Alpha tocopherol equivalent (mg/kg) [b]	Total natural antioxidants (mg/kg)[c]	DPPH Radical scavenging Activity (%)
RBO (100%)	2803.0±1.8	165.3±1.1	2968.3±1.9	53.9±0.5
Ratio 80:20				
RBO+OO	2294.0±1.4	202.5±0.0	2496.6±1.5	63.8±0.3
RBO+GO	2290.3±1.2	136.6±0.1	2427.0±1.2	47.1±0.2
RBO+SOO	2294.0±1.3	150.7±0.2	2444.7±1.8	52.3±0.2
RBO+SO	2292.2±1.4	247.3±0.0	2539.5±1.6	56.7±0.1
RBO+MO	2291.3±1.9	168.3±0.3	2459.6±1.4	53.6±0.2
RBO+PO	2289.4±1.2	138.9±0.0	2428.3±1.9	49.8±0.1
Ratio 70:30				
RBO+OO	2275.5±1.6	249.5±0.4	2525.0±1.2	67.7±0.3
RBO+GO	2271.8±1.1	134.8±0.1	2406.6±1.8	51.0±0.2
RBO+SOO	2275.5±1.9	159.3±0.2	2434.8±1.2	56.2±0.2
RBO+SO	2273.6±1.6	295.1±0.1	2568.7±1.4	60.6±0.1
RBO+MO	2272.7±1.2	176.6±0.2	2449.3±1.3	57.5±0.1
RBO+PO	2270.8±2.5	123.2±0.1	2394.0±1.4	53.7±0.3

Values are expressed as mean ±SE; the given values for DPPH scavenging activity are after 60 min of incubation.
RBO rice bran oil, OO olive oil, GO groundnut oil, SOO soybean oil, SO sunflower oil, MO mustard oil, PO palm oil
Correlation coefficient: [a]Oryzanol and DPPH scavenging activity =Non significant, [b]Alpha tocopherol equivalent and DPPH scavenging activity =0.75 ($p \leq 0.01$), [c]Total natural antioxidants and DPPH scavenging activity= 0.76 ($p \leq 0.01$)
CD: Oryzanol – between ratio 80:20 and 70:30 = 1.88 ($p \leq 0.05$), Alpha tocopherol equivalent – between ratio 80:20 and 70:30 = 2.14 ($p \leq 0.05$).

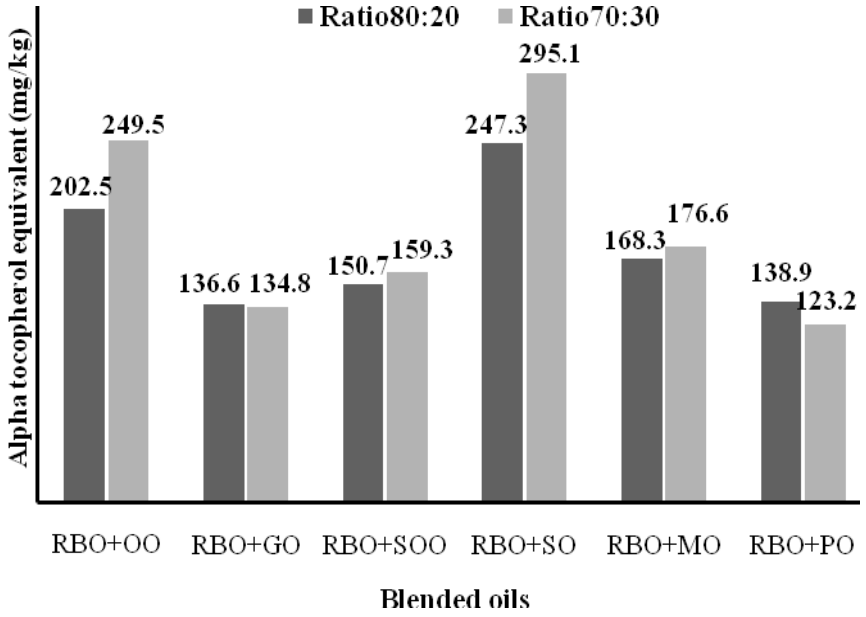

Figure 1 Alpha Tocopherol equivalent content of blended oils of both ratios

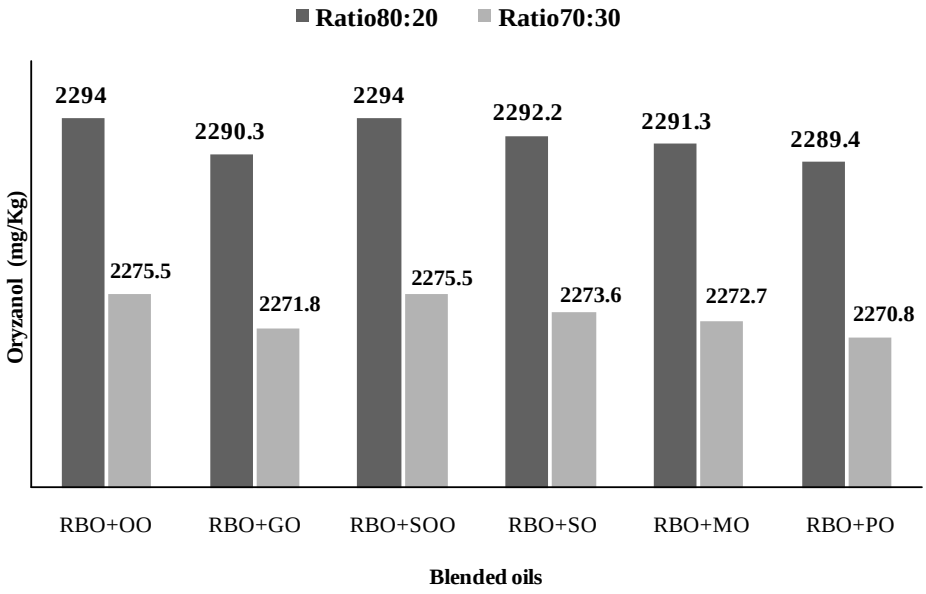

Figure 2 Oryzanol content of blended oils of both ratios

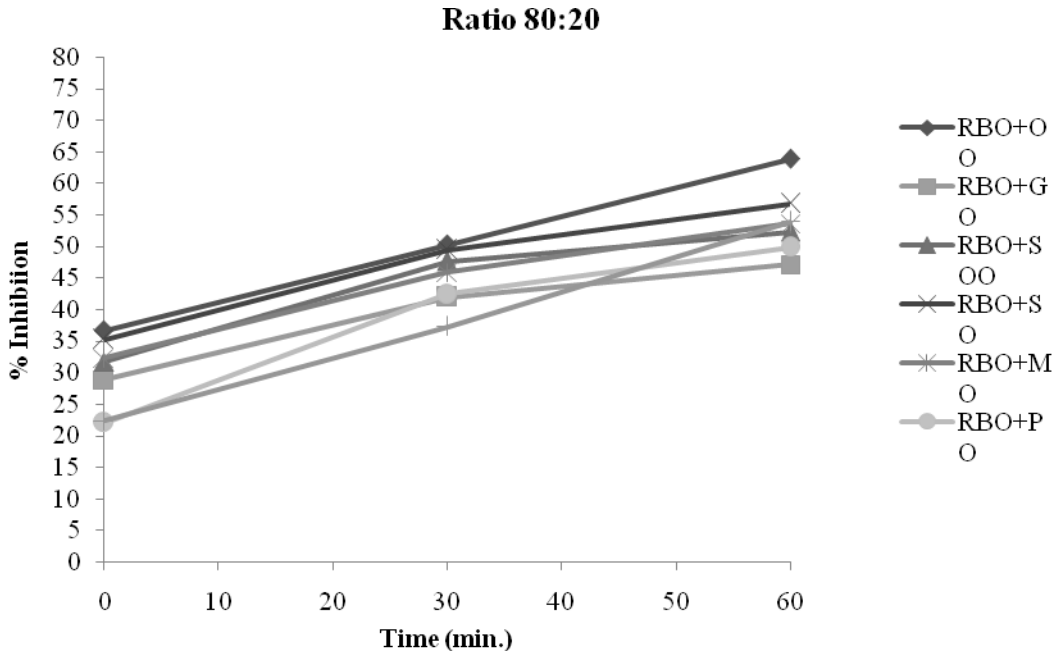

Figure 3 DPPH radical scavenging activity of blended oils (80:20). CD value between time intervals (0, 30, 60 minutes) - 0.26 (p≤0.05), CD value between blended oils - 0.36 (p≤0.05)

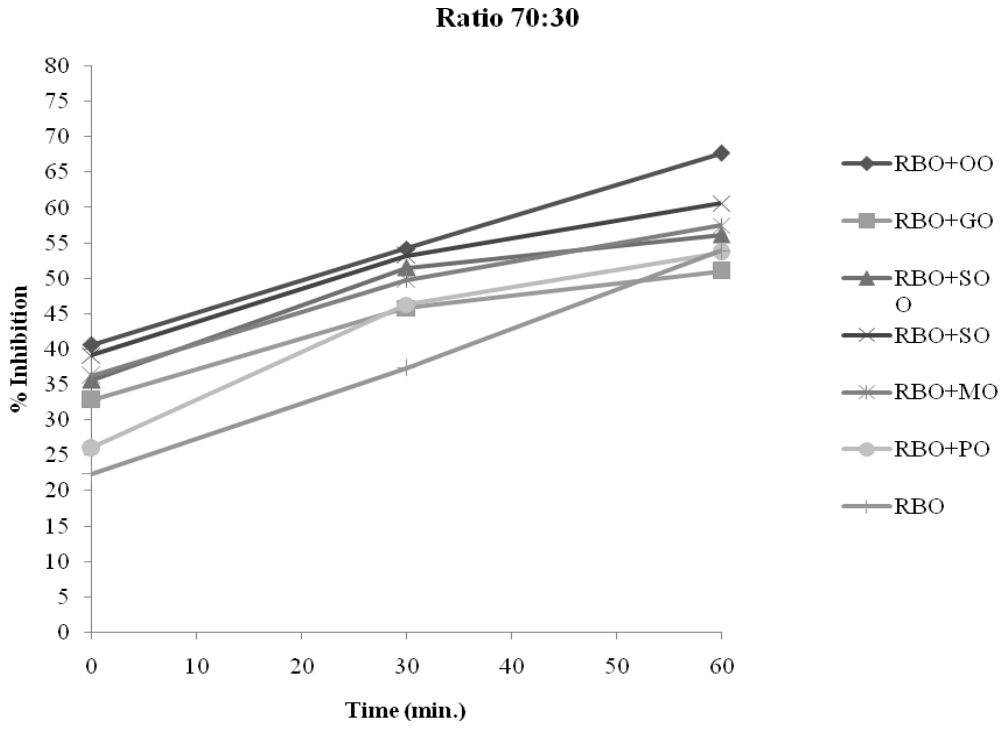

Figure 4 DPPH radical scavenging activity of blended oils (70:30), CD value between time intervals (0, 30, 60 minutes) - 0.31 (p≤0.05), CD value between blended oils- 0.43 (p≤0.05)

was 202.5 and 249.5 mg/Kg respectively. Oryzanol consists of a mixture of ester compounds and has technological usefulness e.g. stabilization of vegetable oils at frying temperature and physiological properties such as antioxidant activity (Lloyd et al., 2000; Rubalya and Neelameagam, 2008). Results showed that RBO+OO and RBO+SOO in the ratio of 80:20 and 70:30 had equal amount of oryzanol i.e. 2294.0 and 2275.5 mg/Kg respectively followed by RBO+SO, RBO+GO and RBO+PO in the both ratios (Figure 2). The highest value of total natural antioxidants was observed in RBO+SO (2568.7 mg/kg) and RBO+OO (2525.0 mg/kg) in the ratio of 70:30 (Table 3).

RADICAL SCAVENGING ACTIVITY (RSA)

This assay is based on the reduction of DPPH radicals in methanol which causes an absorbance drop at 515 nm. In this study, the radical scavenging activity towards DPPH free radicals was expressed as percent inhibition. The DPPH free radical scavenging activities of all blended oils are shown in Figure 3 and 4. Ranking order of percent inhibition showed by different blended oils was RBO+OO>RBO+SO>RBO+MO>RBO+SOO>RBO+ PO > RBO+GO after 60 minutes in the both ratios. By comparing both ratios it was found that RBO+OO in the ratio of 70:30 had highest (67.7%) and RBO+GO in the ratio of 80:20 had lowest (47.1%) RSA towards DPPH radicals. Highest RSA of RBO+OO (70:30) could be due to the presence of oleic acid. Research studies have reported that oleic acid is more stable towards oxidation during storage and cooking (Bastida and Sanchez-Muniz, 2001). Besides, natural antioxidants i.e. oryzanol (2294.0 mg/Kg) and alpha tocopherol equivalent (249.5 mg/Kg) were also present in RBO+OO (70:30). Scientific studies reported that higher the alpha tocopherol and oryzanol content, the higher the DPPH scavenging activity would be (Malik et al., 2011; Vorarat et al., 2010).

CORRELATIONS

A positive correlation ($p<0.01$) between total natural antioxidants and DPPH radical scavenging activity implied that antioxidants in blended oils were capable of donating hydrogen atoms to DPPH free radicals. A positive and significant ($p<0.01$) correlation was also observed between alpha tocopherol equivalent and DPPH radical scavenging activity whereas no significant correlation was found between oryzanol and DPPH radical scavenging activity (Table 3).

CONCLUSION

RBO+OO (70:30) and RBO+GO (80:20) contained highest amount of MUFA (oleic acid) i.e. 47.6 and 36.9 per cent respectively. RBO+SOO contained highest percentage of PUFA i.e. 61.6 followed by RBO+MO (59.2 %) and RBO+SO (58.5 %) in the ratio 70:30. Saturated fatty acids (SFA) were found be highest in RBO+PO i.e. 24.5 per cent in the ratio of 70:30. RBO+PO and RBO+OO showed least percent increase in peroxide formation i.e. 21.4 and 30.3 percent in the ratio of 70:30 respectively. RBO+SO and RBO+OO in the ratio of 70:30 had highest content of alpha tocopherol equivalent i.e. 295.1 and 249.5mg/Kg respectively. RBO+OO and RBO+SOO in the ratio of 80:20 and 70:30 had equal amount of oryzanol i.e. 2294.0 and 2275.5 mg/Kg respectively. The highest value of total natural antioxidants was observed in RBO+SO (2568.7 mg/kg) and RBO+OO (2525.0 mg/kg) in the ratio of 70:30. RBO+OO in the ratio of 70:30 had highest (67.7%) and RBO+GO in the ratio of 80:20 had lowest (47.1%) RSA towards DPPH radicals. Overall, RBO+OO (70:30) contained highest amount of MUFA and adequate amount of natural antioxidants. Also, this blend showed less percent increase in peroxide formation and was able to scavenge DPPH radicals to a greater extent.

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