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ORIGINAL PAPER

Stability of Rice Bran Oil in Terms of Oryzanol, Tocopherols, Tocotrienols and Sterols

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Abstract The effect of oryzanol, tocopherols and tocotrienols (tocols) and sterols individually and as combinations of two were analyzed for DPPH radical scavenging activity and antioxidant activity. Oryzanol, tocols and sterols were isolated by using column chromatography and then added at known concentrations in stripped RBO. The results showed that tocol added samples are more stable than with oryzanol and sterol as individual additions. Among them T_1 (sample having 0.04% tocols) is more stable than T_2 (sample having 0.08% tocols). Comparing T_1 and T_2 with control oil, T_1 had a peroxide value almost similar to control oil (T_1, 5.68 mequiv/kg : control oil, 5.52 mequiv/kg) showing the antioxidant activity of tocols even in the absence of other micronutrients. The diene value of both T_1 (4.27) and T_2 (4.03) is lower than control oil (6.14). While analyzing combinations, prevention of oxidation was significantly better for oryzanol and tocols combinations, OT_1 had a peroxide value of 10.57 mequiv/kg, OT_2, 10.42 mequiv/kg when compared to the control sample (52.25 mequiv/kg). Similarly the diene value 5.86 (OT_1) and 7.1 (OT_2), the *p*-anisidine value 53.8 and 63 for OT_1 and OT_2, respectively. The DPPH activity of samples T_2 (0.08% tocols) and OT_2 (sample having 0.8% oryzanol + 0.08% tocols) had a lower IC_{50} on the initial day and the IC_{50} was lowest for T_1 (0.04% tocols) and OT_1 (sample having 1.6% oryzanol + 0.04% tocols) on the final day.

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Introduction

The stability of oil is a significant factor with regard to the nutritional quality of oil [1]. Autoxidation and photo oxidation are two oxidative processes in lipids. Oxidation generates toxic reactive species which affect the biological pathway and leads to degenerative diseases such as cancer [2]. Major reactive species occurring due to oxidation are radicals, which include superoxide, hydroxyl, alkoxyl, etc. and these species are responsible for the propagation of chain reactions. In the course of the chain reaction, secondary oxidation products such as aldehydes are generated. In this context, the value of antioxidant compounds arises. Antioxidants are compounds which either prevent the autoxidation or shorten the radical generating paths in lipids [3]. Many naturally occurring compounds such as thymol, carvacrol, tocopherol, tocotrienols, etc. were reported as antioxidants. Among them, tocopherols and tocotrienols (tocols) were the most active [4]. Vegetable oils are a good source of tocols, including rice bran oil (RBO). The antioxidant activity of tocols and their crucial effect against oxidative reactions has been documented [5]. In addition to tocols, RBO has oryzanol, sterol and squalene, as important micronutrients [6]. Studies have proved that the stability of the oil depends on the nature and amount of antioxidants in RBO.

Many previous reports emphasize blending of RBO to increase stability of other oils [7]. A blend of sunflower oil and RBO in equal volumes was found to have a protective effect due to the presence of various antioxidants in RBO [8]. A fried dough prepared using the blend of RBO and

soybean oil had reduced hydrolytic as well as oxidative rancidity [7]. A decrease in the formation of peroxides in a blend of coconut oil and RBO was reported [9]. In all the reports, the contributions of tocols and oryzanol were analyzed in quantitative terms as well as their inherent nature on oil stability. Inhibition of lipid peroxidation by RBO tocols has also been studied [10]. In addition to tocols, steryl ferulates are a group of compounds in RBO reported as having antioxidant property [11]. The ferulic acid group in steryl ferulates is responsible for the antioxidant activity and the group of ferulic acid esters of triterpene alcohol and sterols are called γ-oryzanol. In RBO, both vitamin E and γ-oryzanol reduce the formation of oxidized by-products. It has also been reported that γ-oryzanol components have greater antioxidant activity than α- and γ-tocopherols and tocotrienols [5].

The effect of oryzanol, tocols and sterols individually and as combinations on the stability of RBO was investigated. The main objectives of the present study were to evaluate the effect of various concentrations of tocols, oryzanols and sterols on the stability of RBO. The objectives of the study were to isolate the tocols, oryzanols and sterols using chromatography and then determine their effects on RBO stability.

Materials and Methods

Materials

Refined rice bran oil (RRBO) was obtained from the Angamali Oil Refinery (Kerala, India). All chemicals and analytical grade solvents were from Ranbaxy Fine Chemicals (New Delhi, India) and HPLC solvents from J.T.Baker (Mexico). Standards of tripalmitin, 1,2-dioleoyl-rac-glycerol, 1-monopalmitoleoyl glycerol, stearic acid, phytosterols (stigmasterol, beta-sitosterol and campesterol) were purchased from Sigma-Aldrich (Steinheim, Germany), tocopherols and tocotrienols were obtained from Calbiochem (Merck, Darmstadt, Germany). Standard compounds of oryzanol (cycloartenyl ferulate and 24-methylene cycloartanyl ferulate) were a gift from Tsuno Rice Fine Chemicals, (Wakayama, Japan).

Isolation of Oryzanol, Tocols and Sterols

A glass column (110 cm length and 4 cm wide) was packed with silica gel of 60–120 mesh (750 g). RRBO (20 g) was adsorbed on silica gel and packed in the column. For the separation of oryzanol, tocols and sterols, the solvents used were different ratios of hexane and diethyl ether (v/v) viz: 100:0, 95:5, 80:20, 70:30, 65:35 and finally washed with methanol (500 mL) and acetone (500 mL). The volume of solvent used, weight of each fraction obtained are presented in Table 1. All the fractions were evaporated and checked by TLC (silica gel 60 coated glass plates 0.2 mm thickness). For the identification of neutral and polar lipids, the plate was developed in the solvent system of hexane:diethyl-ether:acetic acid (80:20:1 v/v/v) and for oryzanol, tocols and sterols, benzene:chloroform (24:2 v/v) and the separated bands were identified using reference standards.

From the TLC observations, the concentrated fractions of tocols, oryzanol and sterols were identified as 80:20 (v/v), 70:30 (v/v) and 65:35 (v/v), respectively, along with minor amounts of acyl glycerols. Therefore, the fractions were again purified by column chromatography. A column with a length of 90 cm and a 2-cm width was chosen and packed with silica of 60–120 mesh. The 80:20 fraction was separated using the solvent hexane and diethyl ether, the ratios used and the fractions separated were 92:8, 86:14, 84:16, 82:18 and 80:20 (v/v). Fractions 70:30 and 65:35 were reseparated using 95:5, 90:10, 85:15, 80:20, 75:25 and 65:35 (v/v) ratios of hexane and diethyl ether. All fractions were evaporated and analyzed by TLC using the solvent system hexane:diethyl ether: acetic acid (80:20:1 v/v/v) and benzene :chloroform (24:2 v/v) described above. The volume of solvent used, weight of each fraction obtained and the percentage of micronutrients in each fraction obtained by chromatography are presented in Table 2.

High Performance Thin Layer Chromatographic (HPTLC) Analysis of Sterols, Oryzanol and Tocopherols

HPTLC analysis was done using a CAMAG HPTLC system (Switzerland) with a Linomat 5 Automatic Sample Spotter, a CAMAG TLC Scanner 3 and the CAMAG "win

Table 1 Isolation of oryzanol, tocols and sterols from RBO by column chromatography

Eluent hexane:diethylether	Volume (mL)	Weight of fraction[a] (g)	Micronutrients (%)		
			Oryzanol	Tocols	Sterols
100:0	1,000	15.20 ± 0.51	–	–	–
95:5	900	2.69 ± 0.12	2	–	–
80:20	1,000	0.12 ± 0.01	18	10	–
70:30	1,000	0.86 ± 0.06	29	0.46	14
65:35	800	0.61 ± 0.03	6	–	24

[a] Mean ± SE, $n = 3$

Table 2 Percentage (by HPTLC) obtained by recolumn chromatography of oryzanol, tocols and sterol rich fractions

Ratio hexane:diethylether	Volume (mL)	Weight of fraction (mg)[a]	Micronutrients		
			Oryzanol (%)	Tocols (%)	Sterols (%)
80:20					
92:08	500	60.00 ± 0.01	–	–	–
86:14	400	20.00 ± 0.85	–	–	–
84:16	400	5.00 ± 0.06	–	–	–
82:18	400	12.20 ± 0.15	–	98.30	–
80:20	400	22.00 ± 0.15	98.18	–	–
70:30					
95:05	500	218.00 ± 0.28	–	–	–
90:10	400	136.00 ± 0.34	–	–	–
85:15	400	144.00 ± 0.16	–	–	–
80:20	400	5.00 ± 0.28	–	80.00	–
75:25	400	253.00 ± 0.41	97.98	–	–
65:35	400	97.00 ± 0.23	–	–	97.52
65:35					
95:05	500	215 ± 0.32	–	–	–
90:10	400	114 ± 0.33	–	–	–
85:15	400	78 ± 0.48	–	–	–
80:20	400	2 ± 0.39	–	–	–
75:25	400	45 ± 0.46	81.33	–	–
65:35	400	151 ± 1.10	–	–	96.02

[a] Mean ± SE, $n = 3$

CATS" 1.3.0 planar chromatography manager software. The plates used were HPTLC aluminium sheets coated with silica gel 60F254 (E. Merck) (0.2 mm thickness) and the Chamber, CAMAG glass twin trough chamber (10 × 10 cm). Quantification of oryzanol, tocols and sterols was done using a previously standardized method [12]. The method in brief is as follows: the fractions are obtained by recolumn dissolved in chloroform and spotted in HPTLC plates and developed using the solvent system benzene:chloroform (24:2 v/v) in a twin trough chamber, followed by scanning at wavelengths 206, 297 and 325 nm for sterols, tocols and oryzanol, respectively, using a CAMAG TLC Scanner 3. The quantification was done by calculating the area of the corresponding standards of the samples.

Antioxidant Activity of the Bioactive Compounds in RBO

Sterols, oryzanol and tocols were added to stripped oil (column separated oil) at two different concentrations individually and also as mixtures of combinations of two as shown in Table 3. Micronutrients added in each 30 g of stripped oil weighed in glass bottles (50 ml) and the samples were sonicated for 30 min at 35 °C. Two individual concentrations of oryzanol O_1, O_2 at the rate of 100 and 50%: two combinations of oryzanol and tocols, OT_1, OT_2: two combinations of oryzanol and sterol OS_1, OS_2 were prepared and analyzed. Similarly two individual concentration of tocols and sterols, T_1, T_2 and S_1, S_2 at the rate of 50 and 100%, respectively and two combinations of tocols and sterols TS_1 and TS_2 were also analyzed. In order to study the dose-dependent–stability relationship, various concentrations (50 and 100%) were selected. Combinations were selected to compare their effect to individual additions. The micronutrients added to samples were the calculated amounts of total micronutrient originally present in the oil taken for analysis. In order to study the stability parameters, a Schaal oven test was conducted for 5 days. The samples were heated in an oven at 60 °C and analyzed every day for their peroxide values [13], *p*-anisidine values [14] and diene values [15]. Samples with (control oil) and without micronutrients (control sample) were also kept under the same conditions for the analysis.

DPPH Radical Scavenging Activity

The radical scavenging activity of the samples (first and fifth day) were analyzed by DPPH assay [16]. The oil samples of 0–40 mg (before heating) and 0–60 mg (after heating) were made up to 3 mL by adding ethyl acetate after adding 1 mL of 0.01 mM DPPH in ethyl acetate. The decrease in absorbance was determined at 517 nm after 10 min. A DPPH solution was used as the control sample

Table 3 Percentage of micronutrients added individually and as combinations to stripped RBO

Micronutrient	Individual mixing		Micronutrients	Combinations	
	% of mixing	Sample code		% of mixing	Sample code
Oryzanol (O)	1.60	O_1	Oryzano + tocol	1.60 + 0.04	OT_1
	0.80	O_2	O + T	0.80 + 0.08	OT_2
			Oryzanol + sterol	1.60 + 0.60	OS_1
			O + S	0.80 + 1.20	OS_2
Tocols (T)	0.04	T_1	Tocol + sterol	0.08 + 0.60	TS_1
	0.08	T_2	T + S	0.04 + 1.2	TS_2
Sterol (S)	0.60	S_1			
	1.20	S_2			

without oil and as the blank. The % DPPH RSA was calculated as follows:

$$\%\ \text{DPPH RSA} = [(\text{control absorbance} - \text{oil absorbance})/(\text{control absorbance})] \times 100$$

The % DPPH RSA was plotted against the concentration of the oil sample (milligrams) and the IC_{50} value was calculated.

Statistical Analysis

All measurements were duplicated on replicate samples following the same mixing (2 × 2). The results were statistically analyzed by analysis of variance (ANOVA) and Duncan's multiple range test (DMRT). The statistical significance was accepted at a level of $P < 0.05$ [17].

Results and Discussion

Effect of Oryzanol on the Stability of RBO

Oryzanol is the major bioactive phytochemical in RBO having various biological effects. Figures 1, 2 and 3 show the peroxide, diene and *p*-anisidine values.

The 5-day storage studies of RBO with added oryzanol and its combinations at 60 °C, showed variations in the stability values. The control oil (with micronutrients) showed a peroxide value ranging from 7.7 to 5.5 mequiv/kg from the start to the final day and that of the control sample (without micronutrients) 8.1–52.2 mequiv/kg, respectively. Comparing these values with oryzanol samples, O_1 and O_2 does not show any concentration dependent lowering of oxidation but O_2 samples had lower peroxide values, from the initial to the fourth day. Combinations of oryzanol and tocols at the 0.04% tocols (OT_1) level had lower peroxide values from the initial to the fourth day compared to samples containing tocols of 0.08% (OT_2). The lower peroxide values of oryzanol and tocols compared to combinations of O_1 and O_2 may be due to the balanced action of tocols and oryzanol against the oxidation of lipids. Comparing samples O_1 and OT_1 (having 1.6% oryzanol), peroxide value of OT_1 was lower than O_1, which was also observed for O_2 and OT_2. This indicates that they had an additive effect for the OT combinations. In the case of combinations of sterol and oryzanol, OS_1 and OS_2 had high peroxide formation when compared with O_1 and O_2. The explanation for this was that sterol molecule is very susceptible to radical formation, which would favor the radical chain reactions [18]. The peroxide formation was lower in OS_2 (17.3 mequiv/kg) than in OS_1 (41.8 mequiv/kg) except on day one. It was inferred that the addition of tocols to oryzanol, decreases the formation of peroxides when compared to the value of the control sample, O_1 and O_2. Compared to tocols, oryzanol has less effect on radical suppression [19]. However, there were additive effects between oryzanol and tocols, which was substantiated by analyzing the values of the control sample, O_1 and O_2 (52.3 mequiv/kg).

Diene values measure the conjugated double bonds formed during peroxide formation. The hydrogen abstraction from the allylic position of the lipid causes the formation of stable allylic radicals. Diene values of O_1 and O_2 are very different, O_2 had a lower value compared to the control oil. Diene formation in OT_1 and OT_2 decreased until the third day but when analyzing the initial to the final day, OT_1 had a lower diene value than OT_2 this agrees with the lower peroxide formation in OT_1 than in OT_2. The combinations of oryzanol and tocols in general had lower conjugated diene values. However, O_2 did have a lower diene value than tocol combinations. This agrees with a previous report that oryzanol was more effective than tocopherols (alpha) in inhibiting the formation of conjugated dienes [20]. Comparing sterol and oryzanol combinations, OS_1 and OS_2 had higher conjugated diene values than O_1 and O_2 on the final day but they had almost

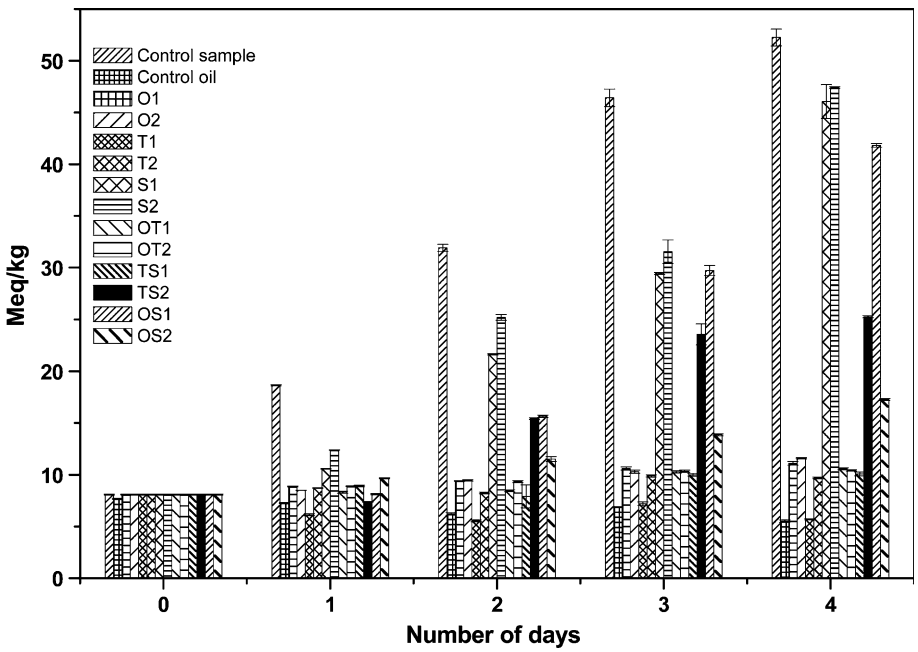

Fig. 1 Effect of oryzanol, tocols and sterols at various concentrations and combinations on the peroxide value of stripped RBO by the Schaal oven method at 60 °C

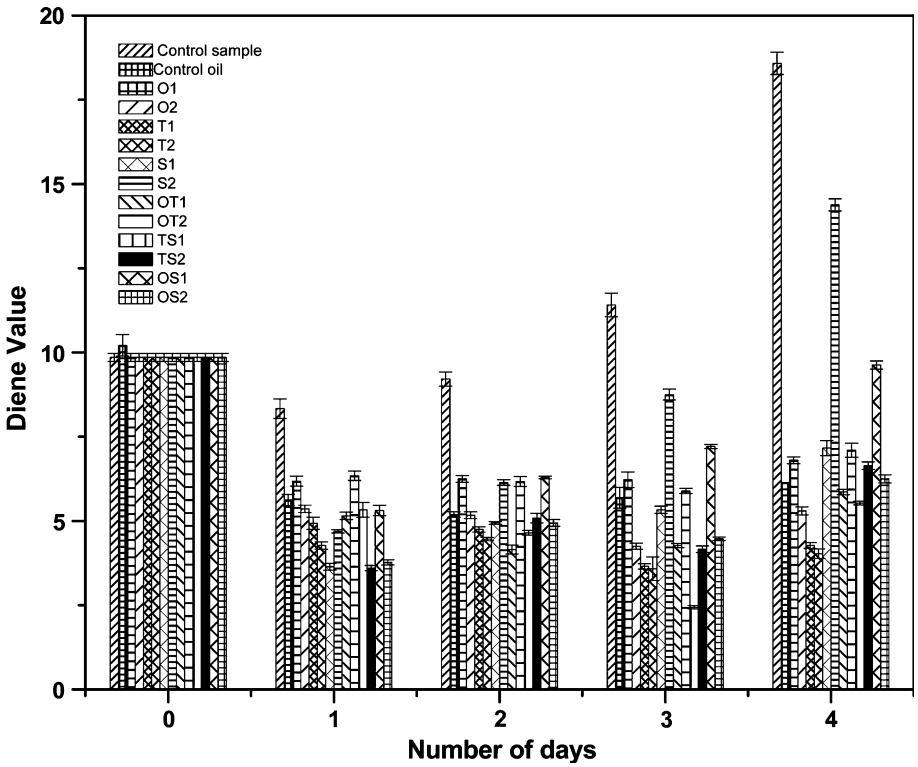

Fig. 2 Effect of oryzanol, tocols and sterols at various concentrations and combinations on the diene value of stripped RBO by the Schaal oven method at 60 °C

comparable values up to the third day, which indicates that sterols neither improve the activity of oryzanol nor do they have antioxidant activity. However, sterol and oryzanol combinations had lower conjugated diene values than the control sample.

The secondary oxidation products were analyzed by the *p*-anisidine values. The control oil had a *p*-anisidine value of 24.4 while that of the control sample reached 162.9. In oryzanol combinations, OT_1 had a lower *p*-anisidine value throughout the analysis. Individual mixing of oryzanol O_1 and O_2 with stripped oil had a *p*-anisidine value greater than that of OT_1 and OT_2, which indicates that oryzanol and tocol combinations reduces the decomposition of hydroperoxides. Among the oryzanol combinations, OT_1 had a lower *p*-anisidine value compared to the control oil. In oryzanol and sterol combinations, OS_2 had lower

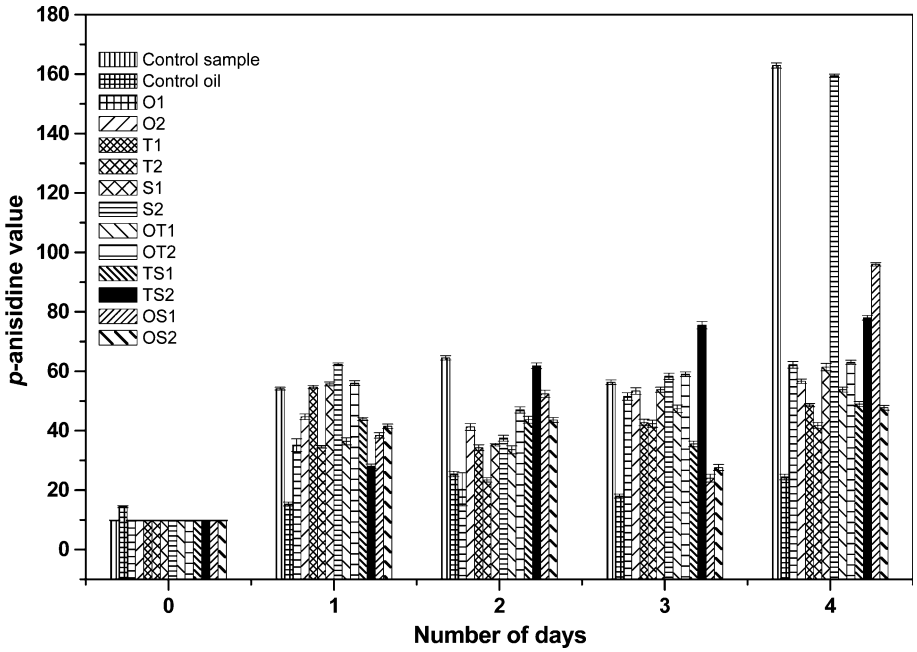

Fig. 3 Effect of oryzanol, tocols and sterols at various concentrations and combinations on the *p*-anisidine value of stripped RBO by the Schaal oven method at 60 °C

p-anisidine values on alternate days with OS_1 and OS_1 had the highest *p*-anisidine value (96.0) among the oryzanol combinations. However, oryzanol combinations OT_1 (53.8) had a lower value than oryzanol individual mixing (O_1: 62.2, O_2: 56.8) with stripped RBO. From the whole analysis, the values are well correlated to each other and as a conclusion among all the samples, the highest stability was found with in tocol combinations and the order of stability was $OT_1 > OT_2$.

Effect of Tocols on the Stability of Oil

Tocols mentioned in the present study includes both tocopherols and tocotrienols. Previous reports [11, 21] mentioned the antioxidant activity of tocols in RBO and in blends of RBO. Figures 1, 2 and 3 show the oxidation values of various samples.

Tocols ranges from 0.04 to 0.08% in refined RBO, which is a major antioxidant responsible for the stability of RBO. Two concentrations of tocols, 0.04% (T_1) and 0.08% (T_2) were added to RBO. T_1 had greater effect in lowering the peroxide formation than by T_2 as evidenced from the first day peroxide values, i.e. 6.1 and 8.7 mequiv/kg, respectively (Fig. 1). The current study supports the previous report which mentions that activity of tocols is higher at lower concentrations [22]. From Fig. 1, it was evident that the peroxide value of T_2 (final day) (9.7 mequiv/kg) was almost double compared with T_1 (5.7 mequiv/kg). In combinations, even though OT_1 (0.04% tocols) had same tocols concentration as in T_1, the peroxide value of OT_1 was nearly double (10.6 mequiv/kg). But when comparing with T_2 the values were almost the same throughout the analysis. Comparing the positive control sample with combinations, oryzanol and tocol combinations had significantly less oxidation. Apart from these results, tocols had more effect than combinations with oryzanol as was evidenced from the values of T_1. Sterols do not suppress the oxidation reactions and also do not enhance the activity of tocols [23], which was evident from the first day values, T_1 with TS_2. However, the sample TS_1 had comparable values with T_2. As the concentration of sterol increased, the activity of tocols became suppressed and so the peroxide value of TS_2 increased which is evident from the values of T_1 and T_2 with TS_1 and TS_2. TS_2 had tocols in the same concentration as that of T_1 had peroxide values ranging from 8.0 to 25.3 mequiv/kg in the initial and final days, respectively. This effect can be explained according to a previous report that sterols having an ethylidene group in the side chain has an antioxidant property rather than stigmasterol, β-sitosterol and campesterol, which are in greater amounts in RBO. The mechanistic aspects behind this is that the free hydrogen atom in the allylic carbon of the ethylidene group is more prone to radical formation and this radical isomerizes to a tertiary radical and becomes more stable. But in the case of stigmasterol, β-sitosterol and campesterol even though a tertiary radical was formed it does not exhibit antioxidant activity due to some steric effects [24]. The overall effect of individual and combinations of tocols on the stability of RBO decreases in the order $T_1 > T_2 > OT_1 > OT_2 > TS_1 > TS_2$ based on the final day values.

Diene formation in both T_1 and T_2 was less than in the control oil (Fig. 2). This shows the greater antioxidant activity of tocols even in the absence of other micronutrients.

Final day diene values of samples having the same concentration of tocols viz: T_2 (4.0) and OT_2 (7.1), T_1 (4.3) and OT_1 (5.9) showed increased diene formation than do tocols individually mixed. Samples of sterol combinations showed comparatively lower diene values than tocol combinations. The oils with tocols and oryzanol combinations had lower peroxide values than sterol combinations. This observation indicates that initially diene formation is greater by abstracting hydrogen from lipid, however further propagation of radicals was arrested due to the strong antioxidant activity of oryzanol and tocols.

Secondary oxidation products were measured by *p*-anisidine value. The control sample had very high *p*-anisidine value compared to other samples indicating rapid formation of secondary oxidation products in the absence of antioxidant compounds. The *p*-anisidine values of T_1 and T_2 were 48.6 and 41.7, respectively (final day), which was much lower than the control sample (162.9) but higher than the control oil (24.4). The T_1, OT_1 and T_2, OT_2 have *p*-anisidine values comparably lower (Fig. 3), due to the activity of tocols. Tocols individually and in combinations, i.e. T_1, OT_1 and OT_2, have a significant effect on the stability of oil, among them T_1 had values similar to the control oil, i.e. a peroxide value of 5.7 and 5.5 mequiv/kg for the T_1 and control oil, respectively; diene values of 4.3 and 6.1 for T_1 and control oil; and *p*-anisidine values of 48.6 and 24.4 for T_1 and control oil, respectively.

Effect of Sterol on the Stability of Oil

Stigmasterol, campesterol, betasitosterol are the major sterols in RBO which have diverse effects on the stability of oil. Previous reports showed the stigmasterol did not acting as an antioxidant but Δ^5 avenasterol are effective antioxidants [24]. The present investigation focused on the stability effects of sterol in stripped RBO by adding two concentrations viz: 0.6 and 1.2% and as combinations with tocols and oryzanol. From the peroxide values, sample S_1 (46.1 mequiv/kg) and S_2 (47.0 mequiv/kg) had values nearer to the control sample (52.2 mequiv/kg) on the final day which means that sterols had no effect against the suppression of peroxide formation. While correlating the combinations, from Fig. 1, tocol combinations with sterol showed lower peroxide values than S_1 and S_2. Among the tocol combinations, TS_2 (25.3 mequiv/kg) had a greater peroxide value than TS_1 (10.1 mequiv/kg). Comparing OS_1 and OS_2 with that of TS_1 and TS_2 (final day), the order of activity was $TS_1 > OS_2 > TS_2 > OS_1$. In conclusion TS_1 had a lower peroxide value among the sterol combinations.

Diene values of TS_1 and OS_2 were less and hence in agreement with the peroxide value and the increasing order of diene values was $TS_1 < OS_2 < TS_2 < OS_1$. The diene value of S_2 (14.4) was greater than any other sterol samples. The order of diene values was as follows TS_1 (5.5) $< OS_2$ (6.2) $< TS_2$ (6.6) $< S_1$ (7.2) $< OS_1$ (9.6) $< S_2$(14.4), which indicates combinations with oryzanol and tocols were better than the sterol samples alone.

Sterol has no significant effect on lowering oxidation, which was evidenced from the diene values, peroxide values and from the evaluation of *p*-anisidine. Final day analysis showed S_2 had a *p*-anisidine value very close to the control sample indicating that sterols had no effect against lipid oxidation and its value reached a maximum of 159.5. Comparing S_2 (159.5), with TS_2 (78.0) and OS_2 (47.7): S_1 (61.4) with TS_1 (49.0) (same sterol concentration), the lower *p*-anisidine values indicate the antioxidant effect of tocols and oryzanol in combinations.

Total Radical Scavenging Assay (DPPH)

The antioxidant activity of oryzanol, tocopherols and sterols in their combinations and as individual additions were analyzed using 0.1 mM solution of DPPH. The IC_{50} values of samples at the time of mixing and after conducting the Schaal oven test were analyzed. The control oil showed IC_{50} value of 5.53 mg on the initial day. In individual mixing of oryzanol (O_1 and O_2), tocols (T_1 and T_2) and sterols (S_1 and S_2), O_2 (0.8%) and T_2 (0.08%) showed almost the same antioxidant activity on first day analysis (Fig. 4). From the results, it was evident that oryzanol had DPPH radical scavenging activity compared with tocopherols at particular concentrations [19]. O_2 and O_1 had varied effects on antioxidant activity on the initial and final days which supports the previous reports showing that the effect of oryzanol on stability depends on the concentration [25]. The samples T_1 and O_1; T_2 and O_2 had similar values on initial day analysis. The order of the IC50 values on the initial day of analysis was $T_2 > O_2 > T_1 > O_1$. Sterols had no significant effect on the IC_{50} value (21.17 mg), reaching a maximum among all samples. The IC_{50} values do not differ much initially for samples with similar tocol and oryzanol concentrations. In combinations, OT_2 had an IC_{50} value of 10.13 mg, which was significantly lower compared with other combinations on the first day at the time of mixing. IC_{50} value of OT_2 was less than O_1, O_2, T_1 and T_2, which strongly infers the additive effect of oryzanol and tocols. Among the tocol and sterol combinations, TS_2 had an IC_{50} value of 9.93 mg. The same trend was observed in oryzanol and sterol combinations having a lower IC_{50} value than individual sterol concentrations in the sample [24].

DPPH activity of the samples was analyzed on the fifth day. In individual mixing of oryzanol (O_1 and O_2), tocols (T_1 and T_2) and sterols (S_1 and S_2), T_1 had an IC_{50} value of 14.24 mg while T_2 had a value of 21.43 mg. This result is supporting evidence for the earlier reports that the tocols had higher activity against radicals at lower concentrations

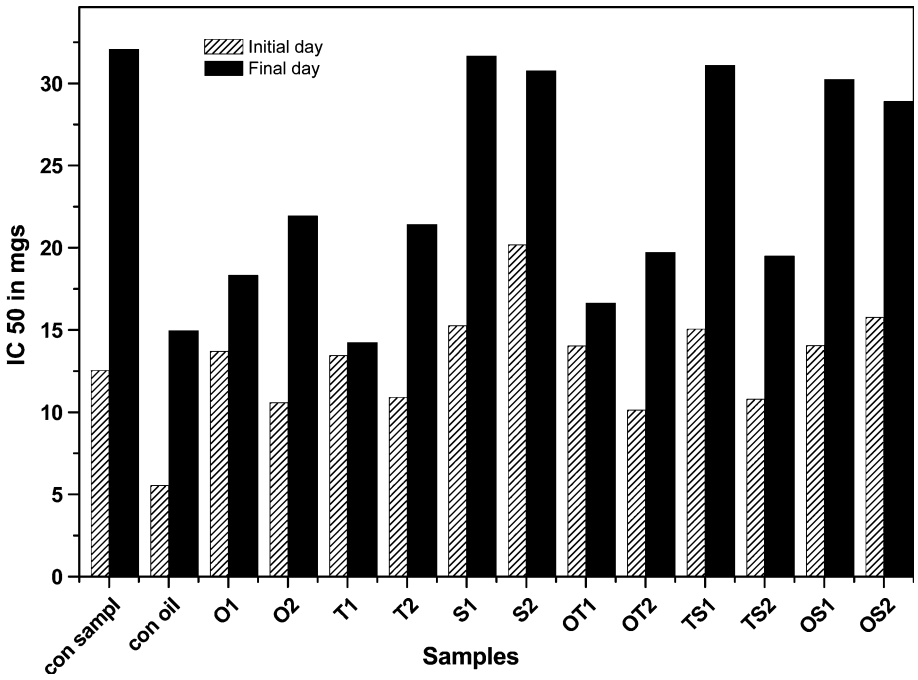

Fig. 4 DPPH radical scavenging effects of stripped RBO with added oryzanol, tocols and sterols at various concentrations and combinations on the initial and final day of mixing

[22]. An important observation was that T_1 had the same IC_{50} value as the control oil on the final day, which was lower than other samples. OT_1 had lower IC_{50} values than OT_2. In general the concentration of tocols in T_1 and OT_1 was the same and hence the antioxidant effect showed by the sample had a comparative effect.

Conclusion

This research confirmed that among the individual concentrations of all micronutrients, T_1 had the greatest effect followed by T_2 while sterol and oryzanol had minimal activity. Among the combinations, OT_1 had a greater effect than OT_2 which is evidence that an additive effect of oryzanol and tocols is involved in RBO stability.

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