Oryzanol as natural antioxidant for improving sunflower oil stability

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Abstract Sunflower oil is being made shelf stable by the incorporation of synthetic antioxidants such as tertiary butyl hydroquinone (TBHQ), while natural antioxidants like oryzanol and tocopherols can also be used. The aim of the current investigation was to evaluate the antioxidant effect of natural oryzanol (Oz) concentrate (15.5 % oryzanol) and purified Oz (80 % oryzanol) on oxidative and thermal stability of sunflower oil. Sunflower oil was incorporated with Oz concentrate to provide 0, 0.12, 0.25, 0.50, 0.84, 1.0, 1.60, 2.0, 2.5 and 3.20 % oryzanol in the oil, stored for 5 weeks at 37 °C and oxidative stability was evaluated. It was found that the oryzanol concentrate showed good antioxidant effect with increase in concentration of oryzanol. In another set of experiments, sunflower oil containing purified Oz at 1 % level individually and in combination with 0.1 % α -tocopherol (α -T) was heated at 120 °C for 24 h to evaluate thermal stability. Sunflower oil containing 1 % Oz (80 % purity) showed 98.40 % and sunflower oil containing 1 % Oz and 0.1 % α -T showed 108.75 % antioxidant effect compared to TBHQ taken as 100 %. The study indicated that sunflower oil containing 1 % Oz (80 % purity) and 0.1 % α -T combination provides a synergistic effect in inhibiting primary and secondary products and showed highest thermal stability. SFO containing 1 % Oz added as concentrate also showed good antioxidant effect during storage. Hence, instead of using synthetic antioxidants like TBHQ, we can add natural oryzanol (purified or as concentrate) to sunflower oil to increase its oxidative and thermal stability.

Keywords Antioxidant/synergistic effect · Natural antioxidant · Oryzanol · Oryzanol concentrate · Sunflower oil

Abbreviations

SFO	Sunflower oil
RBO	Rice bran oil
Oz	Oryzanol
α -T	α -Tocopherol
TBHQ	Tertiary butyl hydro quinone
BHA	Butylated hydroxy anisole
BHT	Butylated hydroxy toluene
PV	Peroxide value
p-AV	para-anisidine value
FFA	Free fatty acid
FAME	Fatty acid methyl ester
MUFA	Mono unsaturated fatty acid
PUFA	Poly unsaturated fatty acid
SFA	Saturated fatty acid

Introduction

During storage of oils, fats and other fat containing foods, lipid oxidation is still a major cause of food quality deterioration, in spite of a wide use of several antioxidants. There are some serious problems concerning the safety and toxicity of BHA, BHT and TBHQ related to their metabolism and possible absorption and accumulation in body organs and tissues (Lindenschmidt et al. 1986). Therefore, the search for preparations of useful natural antioxidants is highly desirable. Abdalla and Roozen (1999) reported that the six different plant extracts showed antioxidant activities that increased the stability of sunflower oil. Oxidative stability of oils is the resistance to oxidation during processing and storage (Guillen and Cabo 2002). Sunflower oil (SFO) is one of the

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most popular vegetable oils in some countries. It is obtained from *Helianthus annuus*, a native of North America and SFO's property is a typical of vegetable triglyceride oil. The minor constituents uniquely present in certain vegetable oils are associated with medicinal qualities and hence are helpful in preventing diseases and promoting health. These include the oryzanol (Oz) present in rice bran oil (RBO), which has been shown to have hypocholesterolemic activity (Nicolosi et al. 1991; Vissers et al. 2000; Seetharamaiah and Prabhakar 1986).

Chemically, oryzanol is a mixture of ferulic acid (4-hydroxy, 3-methoxy cinnamic acid) esters of triterpene alcohols and plant sterols, known also as ferulates (Gopala Krishna et al. 2001; Gopala Krishna 2002). The major components of oryzanol were found to be cycloartanyl ferulate, 24-methylene cycloartanyl ferulate, campesterol ferulate, β -sitosterol ferulate and cycloartenyl ferulate although eight compounds have been reported (Xu and Godber 1999). Kaimal (1999) has summarized the beneficial health effects of Oz. One application of Oz in pharmaceuticals includes hypocholesterolemic activity (Tsushimoto et al. 1991). Oz also is claimed to have a protective role in lipid peroxidation and thus finds applications in sunscreen agents, as an antioxidant and preservative in cosmetics and food preparations, in the treatment of atopic dermatitis, in senile xeroderma, and in the prevention of skin dryness (Kaimal 1999). A safety assessment of oryzanol indicated no genotoxic or carcinogenic activity (Tsushimoto et al. 1991). Because of these beneficial effects of Oz on human health, global interests in the development of facile methods for separating Oz from RBO are being developed. Prasanth Kumar et al. (2012) investigated the utilization of the Oz as Oz concentrate to deliver its health benefits to consumers in the form of functional food. The study was focus on the utilization of Oz concentrate and purified Oz as natural antioxidant to improve oxidative and thermal stability of sunflower oil. This work was conducted in order to assess the effectiveness of natural antioxidant (Oz) compared to synthetic antioxidant (TBHQ) in preventing lipid oxidation on thermal stability of sunflower oil.

Materials and methods

Materials

Physically refined Rice bran oil (RBO) was obtained from M/s AP Solvex Pvt. Ltd., Dhuri, Punjab, India and refined sunflower oil (SFO) was purchased from the local supermarket. α -Tocopherol (α -T) (98 %) was purchased from E. Merck, Germany. Tertiary Butyl Hydro Quinone (TBHQ) was purchased from Loba Chemie, Mumbai. All chemicals and solvents used were of analytical reagent grade.

Methods

Physicochemical properties of refined sunflower oil, concentrate and purified oryzanol incorporated sunflower oil

The refined sunflower oil was analyzed for quality parameters like peroxide value (PV), free fatty acid value (FFA), saponification value, unsaponifiable matter, iodine value and color. The oil was free of synthetic antioxidants, but contained initially 50 mg of tocopherols/100 g of oil. In spite of containing natural tocopherols, the commercial refined sunflower oil (6 different brands) showed poor oxidative stability. Free fatty acids value, peroxide value, saponification value and unsaponifiable matter were determined by AOCS methods (1997). The color of the samples was determined using a Lovibond tintometer in a 1-inch cell on the Lovibond scale in transmittance mode and expressed as (SR+Y) units. The p-anisidine value (p-AV) is a measurement of carbonyl content in the oils or fats, and was determined by the standard method according to AOCS (1998). It is based on the reactivity of the aldehyde carbonyl bond on the p-anisidine amine group, leading to the formation of a Schiff base that absorbs at 350 nm. About 2 g of the sunflower oil samples were dissolved and made up to 25 ml using isooctane and absorbance (A1) of this fat solution was measured at 350 nm against a blank of isooctane. An aliquot (5 ml) of this solution, respectively 5 ml of isooctane (as blank) was transferred to each of two test tubes of 10 and 1 ml anisidine solution (0.25 % g/v glacial acetic acid) was added to each. After 10 min, the absorbance (A2) was measured at 350 nm against isooctane containing p-anisidine.

Preparations of oryzanol concentrate from physically refined rice bran oil

Oz concentrate was prepared from physically refined RBO according to a recently developed process (Indian patent pending). Rice bran oil was processed in such a way to get an Oz enriched fraction which upon further processing yielded Oz concentrate having 15–20 % Oz. This was further refined and was used to prepare Oz incorporated SFO.

Preparation of oryzanol concentrate incorporated sunflower oil

Natural Oz concentrate prepared in the laboratory (0.97, 1.93, 4.84, 7.74, 9.68, 14.50, 19.34, 24.17 and 29.01 g) was taken in 30 beakers of 250 ml capacity to provide a concentrate of 0.1–3.0 % oryzanol in the sunflower oil. A small amount of SFO was also added to these beakers. Then both of these were mixed well using a magnetic stirrer at 65 °C till the contents become clear. Afterwards, SFO was added to make up a total weight of 150 g and then the contents were mixed on the

magnetic stirrer for 30 min. The level of blend was 150 g and the oryzanol (%) in the 30 beakers were 0, 0.12, 0.25, 0.50, 0.84, 1.0, 1.60, 2.0, 2.5 and 3.20 % respectively. 30 mg of TBHQ and 150 g of SFO were also taken in three separate beakers and mixed well with the help of magnetic stirrer at 65 °C. The samples which contain TBHQ and 0 % Oz separately served as positive control and control sample respectively.

Purification of oryzanol from oryzanol concentrate

The Oz concentrate containing 15.5 % Oz was a gummy material and it contained phospholipids up to 5 %. Two different solvent treatments were carried out on the oryzanol concentrate. The first treatment was carried out to remove phospholipids with hot acetone treatment (80 °C) in the ratio of 1:3 (Oz concentrate: acetone, w/v). Then the mixture was cooled to room temperature (30 °C) for 2 h and the phospholipids were separated by vacuum filtration, and filtrate was desolventised using rotavapor (Rotavapor RE 121A, Buchi, Switzerland) at 45 °C. In the second solvent treatment, the solvent free material (27 % of Oz) (100 g) was mixed with hexane: water in the ratio of 10:1 (300 ml/30 ml). The mixture was kept at −20°C for 48 h for the crystallization of Oz. The crystallized material was separated through vacuum filtration and air dried materials ground into fine powder. The 80 % purity of Oz was attained by this method and the recovery of Oz was up to 65 %. The purified Oz (>80 % purity) was used for the thermal stability evaluation of the Oz incorporated SFO.

Preparation of purified oryzanol incorporated sunflower oil

The purified Oz was weighed in beakers of 250 ml capacity according to the required weight. A small amount of SFO was also added to these beakers. Then both the oils were mixed well using a magnetic stirrer at 65 °C. After the oils in all the beakers were mixed well and purified Oz was melted then again SFO was added to make up a total weight. In the other beaker, TBHQ was added as an antioxidant to SFO and mixed well with the help of magnetic stirrer at 65 °C. This one which had TBHQ added acted as a positive control sample. 15 ml of all these samples in triplicate were transferred to petri plates (1.5 cm height×5 cm diameter) and placed in an oven with air draft at 120 °C. Samples were withdrawn at 2, 4, 6, 8, 12, 16, 20 and 24 h intervals and analysed for PV, p-AV, and fatty acid composition.

Free fatty acid value

Free fatty acid value was analyzed according to method Ca 5a-40 (AOCS 1998). However, oryzanol also reacts with alkali and provides a higher FFA value due to both oleic acid and

oryzanol. To differentiate between the two, the real FFA ascribed to only oleic acid was calculated according to Gopala Krishna et al. (2006a) and expressed as percent oleic acid. The following formula was used to calculate the real FFA content.

$$\text{Real FFA value} = \text{Total FFA value (estimated)} - (\% \text{ oryzanol content} \times 0.425) \text{ (2006a)}$$

This procedure was adopted as there is interference from oryzanol upon the determined FFA values.

Fatty acid composition

Fatty acid methyl esters (FAME) of the oil samples were prepared by transesterification, according to AOCS Method No: Ce 1–62, (1998). FAMES were analysed on a Fisons 8,000 series gas chromatograph (Fisons Co., Italy), equipped with a flame ionization detector (FID) and a fused silica capillary column (100 m×0.25 mm i.d.), coated with 0.20 ml SP2560 (Supelco Inc., Bellefonte, PA) as the stationary phase. The oven temperature was programmed from 140 to 240°C at 4°C/min with an initial hold at 140°C for 5 min. The injector and FID were at 260°C. A reference standard FAME mix (Supelco Inc.) was analysed under the same operating conditions to determine the peak identity. The FAMES were expressed as relative area percent.

Oryzanol content

Oz content of Oz concentrate and purified incorporated SFO were determined by a spectrophotometric method by dissolving 0.01 g of the sample in 10 ml of hexane and reading the absorbance at 314 nm in a 1-cm cell using a double beam UV-visible recording spectrophotometer (model UV-1601, Shimadzu Corporation, Kyoto, Japan). The Oz content was calculated by using the extinction coefficient of 358.9 and expressed as g % (Gopala Krishna et al. 2006b).

Oxidative stability study of oryzanol concentrate incorporated sunflower oil

Prepared samples in triplicate were kept in an incubator which was maintained at 37 °C and 60 % RH (Relative Humidity). Samples were withdrawn from storage every 7 days and analysis like PV, FFA, Color and Oz content were determined according to standard methods (AOCS 1998; Gopala Krishna et al. 2006b).

Thermal stability study of purified oryzanol incorporated sunflower oil

The samples were analyzed for their chemical properties like peroxide value, p-anisidine value and fatty acid composition.

All samples were analyzed in triplicate. Prepared samples were kept in an incubator which was maintained at 120 °C. Samples were withdrawn at regular intervals of 2, 4, 6, 8, 12, 16, 20 and 24 h and analysis like peroxide value, p-anisidine value and fatty acid composition were carried out. The stability of oryzanol for the peroxide value was evaluated using the formula below

%Antioxidative Activity

$$= \frac{(\text{PV sample} - \text{PV control})}{(\text{PV TBHQ} - \text{PV control})} \times 100. \text{PV control (free of oryzanol)}$$

Statistical analysis

The studied quality parameters were analyzed in triplicate and the data obtained was expressed as mean±standard deviation. One-way ANOVA was used to calculate significant difference in the parametry studied (Steel and Torrie 1960).

Results and discussion

Initial physico-chemical characteristics of the oil

The initial physico-chemical parameters of sunflower oil, sunflower oil containing oryzanol concentrate and sunflower oil containing 1 % purified oryzanol were evaluated (Tables 1 and 2). These had the peroxide value (2.0 meqO₂/kg oil), free fatty acid value (0.06–0.23 %), saponification value (190–193 mg KOH/g oil), unsaponifiable matter (1.24–17 %), iodine value (131–134 g/100 g oil), color (1–4 units) and oryzanol (0.0–1.0 %). The sunflower oil containing oryzanol concentrate and purified oryzanol were within the specification of sunflower oil as such.

Thermal stability study at 120 °C for 24 h

SFO without TBHQ was mixed with purified natural Oz, standard α-T (0.1 %) and combination of these two at

different ratios from 0.001 to 1 % (Oz). These sets of incorporated oils were heated at 120 °C for 24 h and compared with SFO as such and SFO with TBHQ (200 ppm) for thermal stability.

Peroxide value

Antioxidant efficacy of purified Oz (added at different concentrations from 0.001 to 1.0 %) and α-T were close to that of TBHQ but combination of these two showed an increased antioxidant effect compared to TBHQ during heating at 120 °C for evaluation of thermal stability (Table 3). Although PV showed variations corresponding to the amount of different purified Oz concentrations incorporated to SFO, there was a gradual increase in PV during heating. As expected, the oils showed higher extent of stability during thermal heating ($p < 0.05$). Among different concentrations of purified Oz, at the level of 1 % Oz+0.1 % α-T found to have maximum thermal stability during heating ranging from 2 to 91 meqO₂/Kg of oil compared with SFO mixed with TBHQ (200 ppm) ranging from 2 to 99.7 meqO₂/Kg of oil. Purified Oz alone was also effective i.e. ranging from 2 to 113.5 meqO₂/Kg of oil but a combination of α-T (0.1 % level) showed significant synergistic effect. Juliano et al. (2005) reported the mechanism of antioxidant activity of gamma-oryzanol on oxidative stability of pharmaceutical oils. However, SFO without added synthetic antioxidant showed increase in PV from 2 to 187 meqO₂/Kg of oil during thermal heating showing insignificant antioxidant effect of natural tocopherol present in the starting refined sunflower oil. Kim et al. (2003) showed that samples of refrigerated cooked beef treated with oryzanol and α-tocopherol were effective in improving the stability. The SFO mixed with purified oryzanol also showed similar trend in the quick stability study of heating at 120 °C for 24 h. It was found that to reach a PV of 50, the sunflower oil mixed with purified 1 % Oz took 10 h while sunflower oil mixed with purified 1 % Oz and 0.1 % α-T, it showed an increased effect to 14 h comparable to 16 h for TBHQ treated sample (Table 3 and Fig. 1b).

SFO was also mixed with purified 1 % Oz, 1 % Oz+0.1 % α-T and SFO was mixed TBHQ (200 ppm) separately. These

Table 1 Physico-chemical characteristics of sunflower oil, sunflower oil containing oryzanol concentrate and sunflower oil containing 1 % purified oryzanol

Parameters	Specification for SFO	SFO	SFO with Oz concentrate	SFO with Oz purified
PV (meqO ₂ /kg oil)	2.0±0.01	2.00±0.02	2.00±0.08	2.00±0.06
FFA Value (% oleic acid)	0.15±0.05	0.08±0.01	0.23±0.05	0.06±0.1
Saponification Value (mg KOH/g oil)	188–194±0.1	192±0.01	193±0.08	190±0.05
Unsaponifiable matter (%)	1.5±0.02	1.24±0.02	1.7±0.1	1.57±0.06
Iodine Value (g/100 g oil)	118–141±0.1	134±0.05	133±0.05	131±0.1
Color (5R+Y units)	1–15±0.08	1.0±0.02	4.0±0.05	1.6±0.02
Oryzanol (%)	0.0±0.0	0.0±0.0	1.0±0.05	1.0±0.01

The values with different superscript in the column are significantly different at $P < 0.05$

Table 2 Changes in the free fatty acid content of oryzanol concentrate incorporated sunflower oil during storage at 37 °C

Sample	Incubation period (Days) at 37 °C					
	0	7	14	21	28	35
SFO+0 % Oz	0.14±0.02 ^a	0.14±0.18 ^a	0.14±0.08 ^a	0.14±0.02 ^a	0.15±0.02 ^a	0.15±0.02 ^a
SFO+0.1 % Oz	0.23±0.05 ^a	0.27±0.28 ^a	0.28±0.204 ^a	0.41±0.1 ^a	0.51±0.80 ^a	0.51±0.01 ^a
SFO+0.2 % Oz	0.38±0.03 ^a	0.42±0.00 ^{ab}	0.46±0.01 ^{abc}	0.59±0.2 ^{bc}	0.72±0.28 ^{bc}	0.74±0.04 ^c
SFO+0.5 % Oz	0.49±0.10 ^a	0.54±0.04 ^{ab}	0.60±0.20 ^{abc}	0.78±0.24 ^{bc}	0.95±0.30 ^{bc}	1.00±0.08 ^c
SFO+0.8 % Oz	0.66±0.08 ^a	0.71±0.08 ^{ab}	0.77±0.14 ^{ab}	0.92±0.10 ^{abc}	0.99±0.24 ^c	1.08±0.04 ^{bc}
SFO+1.0 % Oz	0.75±0.22 ^a	0.80±0.04 ^a	0.85±0.30 ^{ab}	1.01±0.06 ^{ac}	1.18±0.08 ^{bc}	1.28±0.10 ^c
SFO+1.5 % Oz	1.19±0.18 ^a	1.22±0.08 ^a	1.25±0.01 ^a	1.29±0.08 ^{ab}	1.37±0.06 ^{ab}	1.47±0.06 ^b
SFO+2.0 % Oz	1.26±0.01 ^a	1.21±0.05 ^{ab}	1.37±0.06 ^{abc}	1.49±0.32 ^{abc}	1.61±0.32 ^{bc}	1.73±0.32 ^c
SFO+2.5 % Oz	1.27±0.04 ^b	1.41±0.25 ^a	1.52±0.04 ^a	1.59±0.24 ^a	1.66±0.24 ^a	1.76±0.30 ^a
SFO+3.0 % Oz	1.43±0.01 ^b	1.56±0.22 ^a	1.70±0.01 ^a	1.75±0.42 ^a	1.79±0.16 ^a	1.83±0.24 ^a
SFO+TBHQ	0.14±0.01 ^a	0.14±0.01 ^a	0.14±0.01 ^a	0.14±0.01 ^a	0.14±0.02 ^a	0.15±0.05 ^a

The values with different superscript in the row shows significant difference at $P<0.05$

sets of mixed antioxidants (both natural and synthetic) in oils were evaluated for thermal stability and samples were withdrawn for analysis at 2, 4, 6, 8, 12, 16, 20 & 24 h interval of time. Peroxide value and p-anisidine value have been determined as the extent of primary and secondary oxidation. Data for concentration of purified 1 % oryzanol and purified 1 % oryzanol+0.1 % tocopherol indicated in the Fig. 1b, reveal that there was a gradual increase in the PV during the course of thermal stability. Among these samples the SFO mixed with purified 1 % Oz+0.1 % α -T was found to have maximum protective effect during 24 h thermal stability (2.27–97.27 meqO₂/kg of oil) ($p<0.05$). The antioxidant efficacy

of both SFO mixed with purified 1 % Oz (2.27–107.08 meqO₂/kg of oil) and purified 1 % Oz+0.1 % α -T (2.27–97.27 meqO₂/kg of oil) were close to that SFO mixed with TBHQ (2.97–105.57 meqO₂/kg of oil). SFO mixed with purified 1 % Oz sample alone showed less antioxidant property but it was close to TBHQ but in combination with α -T showed synergistic effect and proved to be the most effective antioxidant combination in this study than SFO mixed with TBHQ. However, SFO without natural antioxidant (control) showed increase in PV i.e., from 2.27 to 200.4 meqO₂/kg of oil at the end of 24 h of heating at 120 °C. Considering the effect of individual antioxidant, addition of another

Table 3 Antioxidant effect of added purified oryzanol during heating at 120 °C (Thermal stability) in sunflower oil as measured by peroxide value

	The level of antioxidants in oil			Peroxide value		
	Oz (%)	α -T (%)	TBHQ (%)	Incubation period (h) at 120 °C		Time required to reach a PV of 50
				0 h	24 h	
	0.000	0.0	0.00	2.00±0.01 ^a	187±0.02 ^a	7 h
	0.001	0.0	0.00	2.00±0.02 ^a	160±0.08 ^b	
	0.005	0.0	0.00	2.00±0.01 ^a	160±0.10 ^b	
	0.010	0.0	0.00	2.00±0.01 ^a	154±0.02 ^c	
	0.100	0.0	0.00	2.00±0.01 ^a	150±0.00 ^d	
	0.500	0.0	0.00	2.00±0.01 ^a	147±0.08 ^c	10 h
	1.000	0.0	0.00	2.00±0.01 ^a	114±0.10 ^f	
	0.010	0.1	0.00	2.00±0.01 ^a	106±0.10 ^g	
	0.050	0.1	0.00	2.00±0.03 ^a	130±0.08 ^f	
	0.100	0.1	0.00	2.00±0.04 ^a	117±0.20 ^h	
	0.500	0.1	0.00	2.00±0.01 ^a	102±0.24 ⁱ	14 h
	1.000	0.1	0.00	2.00±0.01 ^a	91±0.08 ^j	
	0.000	0.1	0.00	2.00±0.01 ^a	135±0.28 ^k	
	0.000	0.0	0.02	2.00±0.01 ^a	100±0.02 ^l	16 h

The values in the columns with different superscripts are significantly different at $P<0.05$

Fig. 1 Antioxidant effect of oryzanol concentrate added to sunflower oil during storage at 37 °C (a), Added purified oryzanol and α -tocopherol on antioxidant/synergistic effect in sunflower oil at 120 °C as measured by peroxide value (b) and Effect of purified oryzanol and α -tocopherol on antioxidant/synergistic effect of oryzanol added to sunflower oil at 120 °C as measured by p-anisidine value (c)

antioxidant resulted in a dramatic increase in stability of the oil indicating synergism of α -T with oryzanol. TBHQ alone provided as much protection against oxidation and it is possible that at a suitable concentration of these natural antioxidant can be chosen to provide a greater effect on stability.

P-anisidine value

Secondary products of oxidation are generally measured by using the p-anisidine value. During lipid oxidation,

hydroperoxides, the primary reaction products, decompose to produce secondary oxidation products (aliphatic aldehydes (saturated/ unsaturated), ketones, alcohols, acids and hydrocarbons) which are more stable during the heating process, responsible for off-flavors and off-odors of edible oils. In order to ensure a better monitoring of lipid oxidation process in the heating time, the simultaneous detection of primary and secondary lipid oxidation products is necessary. The p-AV is a reliable measurement of the amount of secondary oxidation products (Zhang et al. 2010; De Abreu et al. 2010). The initial

p-AV was in the range of 1.9–2.5 (Fig. 1c) and increased during thermal stability in all the samples. Crapiste et al. (1999) reported that p-AV remained constant at the early stages of oxidation in sunflower oil but then increased sharply following the decomposition of peroxides. Addition of purified 1 % Oz and 1 % Oz+0.1 % α -T resulted in significant decrease in p-AV ($p<0.05$) relative to the control sample. SFO mixed with TBHQ provide the best protection against secondary oxidation of oil samples subjected to thermal stability. The thermal stability for evaluation of antioxidant activity of purified oryzanol and α -T data are in agreement with those reported by Poiana (2012) and Kalantzakis and Blekas (2006) which highlight that the natural extracts showed a significant inhibitory effect against thermal oxidation of refined oils heated at 120 °C.

Fatty acid composition

There was a difference in the fatty acid composition with respect to the content of linoleic acid in the oryzanol concentrate treated oils due to the oil portion being added along with oryzanol (data not presented). However, when purified oryzanol was added, such a change was not noticed. However, it indicated protection of linoleic acid in the oil during heating at 120 °C for 24 h (Table 4). The fatty acid composition showed an almost similar small reduction in the SFO mixed with purified Oz while for control there is a significant 8–10 % reduction in the content of C18:2 after 24 h heating at 120 °C. This indicates that the 1 % purified Oz and combination of 1 % purified Oz+0.1 % α -T showed an antioxidant effect almost equal to that of synthetic antioxidant TBHQ added at 0.02 % level (Table 4).

Oxidative stability during storage

Peroxide value

Peroxides are the primary oxidation products of oxidative rancidity where oxygen reacts with unsaturated fatty acids in

triglyceride in the presence of heat and light. These primary oxidation routes can be inhibited by the intervention of either synthetic antioxidant (TBHQ) or by natural antioxidant like Oz. Thus Oz acts as a free radical quencher. If such an intervention is not provided, both the primary and secondary products of oxidation starts producing peroxides, aldehydes and ketones resulting in rancidity of the oil. Determination of peroxides can be used as oxidation index for the early stages of lipid oxidation (Rehab 2010; Zhang et al. 2010). The peroxide value of SFO mixed with 0–3 % oryzanol as a concentrate was evaluated and shown in Fig. 1a. The results indicate that oils containing different amounts of Oz concentrate were very resistant to oxidation. All the oils had low initial peroxide value. The conformity of the fresh and treated oils according to Indian specification of refined sunflower oil is shown in the Table 1. The PV was rapidly increasing during storage for the samples with less Oz content viz.; 0, 0.1, 0.2, 0.5, 0.8 and 1.0 % when compared to the ones with high Oz content viz.; 1.5, 2.0, 2.5 and 3.0 %. The one mixed with TBHQ (positive control sample) had almost constant PV (14.02–19.94 meqO₂/Kg of oil) during storage period. The PV test gave consistent results, indicating that addition of Oz concentrate (3 %) gave the best protection against oxidation when compared with TBHQ (Fig. 1a). Increasing the percentage of Oz concentrate in the oil increased the stability significantly. With 2.5 and 3 % Oz samples showing almost constant PV over storage period i.e.; the samples with 2.5 % (14.66–21.28 meqO₂/Kg of oil) and 3.0 % (14.08–14.89 meqO₂/Kg of oil) Oz content showed higher oxidative stability when compared to other samples. Nanua et al. (2000) reported that high oryzanol rice bran oil has been given oxidative stability to the whole milk powder.

Free fatty acid content

One of the main chemical degradation pathways of lipids is hydrolysis that can be measured through determination of the FFA content. Table 2 provides the comparative value of FFA in the SFO containing oryzanol concentrate at different

Table 4 Stability in Fatty acid composition of oil samples treated with purified oryzanol and incubated at 120 °C

Samples	C _{8:0}	C _{10:0}	C _{12:0}	C _{14:0}	C _{16:0}	C _{18:0}	C _{18:1}	C _{18:2}	C _{20:0}	SFA	MUFA	PUFA
SFO (0 h)	nd	nd	0.4	0.2	7.7	2.3	23.0	66.2	nd	10.6	23.0	66.2
SFO (24 h)	0.4	0.3	2.9	1.2	8.5	2.6	24.4	59.7	nd	15.9	24.4	59.7
SFO+TBHQ (0 h)	nd	nd	nd	0.1	7.9	2.3	23.0	66.0	0.6	10.9	23.0	66.0
SFO+TBHQ (24 h)	nd	nd	0.3	0.2	8.7	2.4	23.3	65.0	nd	11.6	23.3	65.0
SFO+1 % Oz (0 h)	nd	nd	nd	0.1	7.8	2.3	23.1	66.7	nd	10.2	23.1	66.7
SFO+1%Oz (24 h)	nd	nd	0.1	0.1	8.5	2.5	23.9	64.9	nd	11.2	23.9	64.9
SFO+1%Oz+0.1% α -T (0 h)	nd	nd	nd	0.1	7.6	2.3	23.2	66.7	nd	10	23.2	66.7
SFO+1 % +0.1% α -T (24 h)	nd	nd	nd	0.1	8.1	2.5	24.0	65.3	nd	10.7	24.0	65.3

Values are average of duplicate determinations, *nd* not detected

concentrations (0.1–3.0 %) during 35 days storage at 37 °C. It can be seen that the FFA value was increasing with increasing concentration of Oz concentrate from 0 to 3.0 % Oz content. Also the FFA value was increasing over storage except for 0 % Oz (0.14–0.15 % Oleic acid) content and the one with TBHQ (0.14–0.15 % oleic). No hydrolysis took place in these two as they were not incorporated with Oz concentrate. Hence, the FFA content increased in the SFO mixed with Oz concentrate due to high FFA content in the Oz concentrate itself which increased further during storage. This showed that Oz concentrate addition may increase FFA content of the mixed samples (Gopala Krishna et al. 2006a). When purified Oz was used at 1 %, the FFA content remained similar to that of TBHQ treated oil samples. Hence, it is better to use purified oryzanol and not the concentrate for stabilization of SFO (or other oils).

Color

The color of the SFO was comparable with Oz concentrate mixed with SFO and the data (not presented) shows that the color increases with increasing amount of Oz concentrate in the SFO. Initial value of SFO mixed with Oz concentrate itself showed a higher value ranging from 4.0 to 45.9 with increasing concentration of oryzanol (added as concentrate) from 0.1 to 3.0 %. But the initial value of SFO as such (1.0 unit) and SFO with TBHQ (1.3 unit) showed less color. During the storage period the initial color of treated SFO remained almost constant in all up to the end of 35 days of storage. However, the Oz concentrate mixed with SFO was darker in color compared to SFO as such and SFO mixed with TBHQ. The color value of 45.9 for SFO mixed with 3.0 % Oz content was the highest compared to all others. The color value of 1.0 % purified oryzanol (1.6 unit) mixed oil was better than the oil treated with oryzanol concentrate having similar oryzanol content in the oil. Lighter color of vegetable oils is a very important parameter appealing to the consumers. Hence it is suggested to use purified oryzanol rather than oryzanol concentrate for stabilization of SFO (or other oils).

Stability of oryzanol

The Oz content in the stored oil samples was determined at regular intervals of 7 days for 5 consecutive weeks which ranged from 0.12 to 3.23 %. The initial values were also in the same range which indicated a good stability of the oryzanol level in the SFO mixed with Oz concentrate. This was as expected since oryzanol is stable even at higher temperatures (Gopala Krishna et al. 2005).

Conclusion

Commercial sunflower oil being rich in PUFA has poor stability. Refined vegetable oils generally are being made shelf stable by the incorporation of synthetic antioxidants (TBHQ, BHA, BHT and others). However, natural antioxidants like oryzanol and tocopherol can also be used for improving the stability of PUFA rich edible oils. In order to exploit the potential of Oz as a natural antioxidant on retarding the lipid oxidation process in sunflower oil subjected to thermal and oxidative treatment, this work was undertaken. From the present study on natural oryzanol incorporated SFO it can be concluded that the SFO mixed with 1 % purified oryzanol+ 0.1 % α -T combination showed high oxidative and thermal stability when compared to others and this was due to the synergistic effect of Oz with α -T. Hence, instead of using synthetic antioxidants like TBHQ, we can add natural oryzanol to sunflower oil to increase its oxidative and thermal stability. In conclusion, the observed higher thermal stability of sunflower oil after addition of oryzanol suggests that oryzanol may have potential application as natural antioxidant in the sunflower oil and food industry. Recently Preeti Chandrashekar et al. (2012) reported the serum and liver cholesterol lowering property by oryzanol concentrate in groundnut oil. Thus, it can be used as the stable edible oil to deliver hypocholesterolemic and hypolipidemic benefits to consumers.

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