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# Effect of addition of rice bran oil extract on the stability of sunflower oil, sesame oil and their blends

Bardhan, J., Sahoo, B., Chakraborty, R. and *Raychaudhuri, U.

*Department of Food Technology and Biochemical Engineering Jadavpur University, Kolkata 700032, India*

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**Abstract**

The present study investigates the effect of exposure at elevated temperature (60°C) on the stability of edible oils i.e. sunflower oil, sesame oil and their blends in various proportions of 20:80, 50:50 & 80:20 v/v, with and without addition of antioxidants on the basis of acid value, peroxide value and thiobarbituric acid reactive substances (TBARS) value changes. Tocotrienol rich fraction (TRF) extracted from crude rice bran oil was used as a source of natural antioxidant. Addition of TRF at the optimum level to edible oils was observed to show better stability compared to oils without TRF. The optimum level of TRF activity was measured to be 5 µl/g oil. Considering the combined beneficial effects of sunflower and sesame oil, the blend of these two oils (50:50 v/v) with TRF has been found to be the best. Hence, the stability of edible oil could be enhanced by blending and addition of natural antioxidant.



## Introduction

The stability of edible oil is directly related to its resistance to physical and chemical changes occurring due to lipid peroxidation, hydrolytic rancidity, heating and enzymatic hydrolysis. The most important external variables influencing oil stability towards oxidation are oxygen concentration, temperature, light and metal ions. Besides these, most of the groups of unsaponifiable compounds present in edible oil are reported to have either a beneficial or detrimental effect on oil stability although the primary antioxidants present in the unsaponifiable fraction contribute to the resistance of oil towards oxidation (Velasco and Dobarganes, 2002).

Sunflower oil contains 12% saturated acids (palmitic and stearic), 15–25% oleic acid and 62–70% linoleic acid. It possesses high oxidative stability due to its high content of α-tocopherol (Grompone, 2005). Sesame oil, containing less than 20% saturated fatty acids, 35.9–42.3% oleic acid and 41.5–47.9% linoleic acid is widely used in India for cooking, medicinal and cosmetic purposes (Hwang, 2005). Most of the researchers have found that blend oils at particular ratios retain better oxidative stability as compared to that of sole oils. By blending different types of oils, the consumer can be offered a better quality product with respect to flavor, frying quality and nutritive value (Chopra *et al.*, 2004; Nzikou *et al.*, 2009). It is necessary to ascertain a particular ratio of different oils which can show better oxidative stability upon storage ( Dhawan *et al.*, 2009) .

Antioxidants are used widely in oil to delay the onset of oxidation by quenching lipid and peroxy radicals to convert them to more stable, non-radical products thus maintaining oil stability and improving the shelf life (Giese, 1996). Presently, the use of synthetic antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tertiary butylhydroquinone (TBHQ) is becoming limited in the food industry due to their perceived carcinogenic potential (Jeong *et al*., 2004). On the other hand, plant-derived natural antioxidants are gaining much appreciation due to anti-carcinogenic attributes and other reported medicinal benefits (Iqbal *et al.*, 2007; Sultana *et al.*, 2009). Among natural antioxidants, rice bran oil contains significant amount of tocotrienols (Shin and Godber, 1994) which are very effective in scavenging free radicals to terminate the propagation of free radicals during auto-oxidation thus generating unreactive phenoxyl radicals as well as hydroperoxides (Kitts, 1997). Although α-tocopherol has been historically considered to have the greatest biological activity among the tocochromanol isoforms, tocotrienol has received more attention from researchers recently due to the beneficial properties of α- and γ-tocotrienols such as inhibition of cholesterol synthesis, lowering levels of serum cholesterol (Qureshi *et al*., 2001), physiological potential including antitumor properties towards mammary cancer (Guthrie *et al*., 1997; Nasaretnam, 2005) and free radical scavenging activity (Serbinova *et al.*, 1991). Moreover, the uniform distribution in the membrane lipid bilayer provides a more efficient interaction of the chromanol ring of tocotrienol with lipid radicals as compared to that of tocopherol (Serbinova *et al.*, 1991). Though tocotrienol has been reported to have potent antioxidant activity, it is

*Corresponding author.
Email: *urcfoodtech@gmail.com*

necessary to evaluate the optimal dose at which the antioxidant effects are exhibited maximum because excess amount of tocotrienol rich fraction (TRF) can show pro-oxidant activity (Traber and Packer, 1995; Muid *et al.*, 2013). In this study, the storage stability of pure sunflower, pure sesame oil and their blends has been studied. Tocotrienol rich fraction (TRF) as natural antioxidant was extracted from crude rice bran oil. The storage stability of pure sunflower oil, pure sesame oil and their blends with and without TRF has been examined under the accelerated oxidation condition of exposure at elevated temperature (60°C). The parameters, acid value (AV), peroxide value (PV), thio-barbituric acid reactive substances (TBARS) value have been measured at the interval of 7 days for 42 days.

*Materials and Methods*

Oxalic acid was obtained from SRL, Mumbai. Chloroform ($CHCl_3$), sodium hydroxide (NaOH), acetic acid (CH3COOH), potassium iodide (KI), sodium thiosulphate pentahydrate ($Na_2S_2O_3.5H_2O$), starch, ethanol, butanol and thiobarbituric acid were purchased from Merck Specialities Pvt. Ltd., Mumbai, India. Potassium dichromate ($K_2Cr_2O_7$) and phenolpthalein were supplied by Rankem (Fine Chemicals Ltd., New Delhi). All the chemical reagents and solvents were of highest analytical grade. Sunflower oil and sesame oil were purchased from local market and crude rice bran oil was obtained from local oil mill.

*Preparation of samples and their storage*

Sunflower oil: sesame oil blends were prepared in the proportions of 20:80, 50:50 and 80:20 v/v respectively. The samples S1 (sunflower oil: sesame oil = 100:0), S2 (sunflower oil: sesame oil = 80:20), S3 (sunflower oil: sesame oil = 50:50), S4 (sunflower oil: sesame oil = 20:80) and S5 (sunflower oil: sesame oil = 0:100) were stored at elevated temperature (60°C) in an incubator for 42 days. Oil samples were withdrawn at an interval of 7 days and the parameters such as acid value (AV), peroxide value (PV) and thiobarbituric acid reactive substances (TBARS) value were measured.

*Synthesis of TRF*

The crude rice bran oil was mixed with ethanol at 25°C and kept in a shaker for about 45 minutes. Then the alcohol layer was separated and the process was repeated twice more in succession. The alcohol mixture was evaporated under vacuum at low temperature (40°C) to obtain a fraction that contains many medicinally beneficial micronutrients of which tocotrienol is a major component (Ghosh, 2007).

*Evaluation of optimum level of TRF activity*

To evaluate the optimum level of TRF, different amounts of TRF (2.5 µl/g, 5 µl/g, 7.5 µl/g & 10 µl/g) were added to the oil samples i.e. S1 (sunflower oil: sesame oil = 100:0), S2 (sunflower oil: sesame oil = 80:20), S3 (sunflower oil: sesame oil = 50:50), S4 (sunflower oil: sesame oil = 20:80) and S5 (sunflower oil: sesame oil = 0:100). The peroxide values of these oil samples stored at elevated temperature (60°C) in an incubator have been measured at an interval of 7 days for 21 days. The TRF amount with minimum peroxide values was evaluated as optimum concentration of TRF for these oil samples.

*Addition of TRF to oil samples and their storage*

Pure sunflower (S1′), pure sesame oil (S5′) were taken and sunflower:sesame oil blends were prepared in the proportions of 80:20, 50:50 and 20:80 v/v, marked as S2′, S3′ and S4′, respectively. The TRF was added at the optimum concentration as obtained from the prior experiments (Table 1) to each oil sample and the samples were stored in an incubator at elevated temperature (60°C). The storage stability of these oil samples has been studied for 42 days in terms of acid value (AV), peroxide value (PV) and thio-barbituric acid reactive substances (TBARS) value which have been measured at the interval of 7 days.

*Determination of acid value*

The acid value of an oil or fat is defined as the number of milligrams of sodium hydroxide required to neutralize the free acidity in 1 gm of sample. As rancidity is usually accompanied by free fatty acid formation, the determination of acid value is often used as a general indication of the condition and edibility of oils. The acid value was determined by the AOAC method number 28.030 (Sharma *et al.*, 2006).

*Determination of peroxide value*

The peroxide value was determined according to the AOAC official method 28.024 (Sharma *et al*., 2006). The peroxide value depends on the reaction of potassium iodide in acid solution with the bound oxygen followed by titration of the liberated iodine with sodium thiosulphate.

*Determination of TBARS value*

The TBARS value was determined according to the method described in (Kirk and Sawyer, 1991). The thiobarbituric acid test is based on the color reaction of TBA with malondialdehyde, the secondary oxidation product of lipid peroxidation. The sample mixed with TBA was kept in water bath (95°C),

cooled and measured spectrophotometrically.

## Results

*Optimization of dose of TRF*

Table 1 shows the effect of exposure at elevated temperature (60°C) on peroxide value of pure sunflower oil (S1), pure sesame oil (S5) and their blends S2, S3 and S4 with the addition of TRF at different concentrations of 2.5, 5, 7.5 and 10 µl/g. It was clearly noticed that PV of each oil sample increased with the progress of time of exposure. After 21 days of storage study, the peroxide values for respective oil samples S1, S2, S3, S4 and S5 were measured to be 27.04, 27.95, 27.7, 28.20 and 28.89 meq/Kg; 17.89, 18.23, 17.95, 20.05 and 20.24 meq/Kg; 29.28, 29.97, 30.45, 34.79 and 36.56 meq/Kg and 38.65, 36.28, 39.95, 39.56 and 42.35 meq/Kg at the TRF concentrations of 2.5, 5, 7.5 and 10 µl/g, respectively. Oil samples with a TRF concentration of 5 µl/g showed the least peroxide values after 21 days of storage. Hence, 5 µl/g TRF concentration was evaluated as optimum concentration.

*Effect of exposure at elevated temperature (60°C) on sunflower, sesame oil and their blends*

Figure 1 (a) shows the effect of increasing time of exposure at elevated temperature (60°C) on acid value of the oil samples. Acid value is a measure of the amount of free fatty acids which are liberated by hydrolysis from the oil glycerides. Initially, the acid values of 0.17, 0.30, 0.47, 0.35 and 0.43 mg on 0 day were observed for S1, S2, S3, S4 and S5, respectively. Initially, the steepness of the curve has been found to be high due to higher rate of production of free fatty acids. Then, the slope of the curve was noticed to decrease as the increase in the development of free fatty acids in the samples becomes gradual. The steepness was the maximum for S5, followed by S4, S3, S2 and S1. Finally on 42nd day of storage, the acid values for these samples were observed to be 1.71, 2.90, 4.1, 5.95 and 6.95 mg, respectively. As the time of exposure increases, the AV of all the oil samples have been found to increase due to formation of free fatty acids from the glyceride molecules. However, in case of S3, the AV has been observed to increase at a comparatively lower rate. It has been found that as the amount of sesame oil in the blends increases, the AV also increases correspondingly. The decreasing order of storage stability of these oil samples on the basis of AV was observed to be S1>S2>S3>S4>S5.

Figure 1 (b) describes the effect of exposure at elevated temperature at 60°C on peroxide value of the oil samples. Initially the peroxide values were found to be 7.23, 4.69, 4.73, 4.81 and 7.76 meq/Kg on 0 day for S1, S2, S3, S4 and S5, respectively. The PV of all the samples increases with the progress of time of exposure to 60°C. At the beginning, the sample S4 showed a drastic increase in PV from 4.81 meq/Kg on 0 day to 47.59 meq/Kg on 7th day. The slope for S2 became steeper when a drastic increase was observed in PV from 23.5 meq/Kg on 14th day to 82.35 meq/Kg on 21st day. Initially the rate of increase was found to be low in case of S1, S2, S3 and S5. However, the rate of increase in case of S4 is much steeper initially. Though both sunflower oil and sesame oil contain high amount of linoleic acids (18:1) as major fatty acids, their blends with 80:20 or 20:80 are found to be more prone to auto-oxidation as compared to other samples. This may be due to

Table 1. Effect of TRF at different concentrations on peroxide value of sunflower, sesame oil and their blends

| Concentrations of TRF (µl/g) | Time of exposure (days) | Peroxide value of oil samples (meq/Kg) S1 | S2 | S3 | S4 | S5 |
|---|---|---|---|---|---|---|
| | 7 | 9.34±0.48* | 12.45±0.84* | 11.78±1.05* | 14.82±1.32* | 15.25±1.21* |
| 2.5 | 14 | 15.45±1.04* | 19.25±1.73* | 17.77±1.45* | 20.75±1.27* | 22.43±0.88* |
| | 21 | 27.04±1.80* | 27.95±1.70* | 27.7±2.94$^{NS}$ | 28.20±2.63* | 28.89±2.56* |
| | 7 | 7.23±0.35* | 6.98±0.54* | 4.73±0.42* | 7.65±0.56* | 7.78±0.64* |
| 5 | 14 | 13.65±1.20* | 15.83±1.40* | 16.76±1.28* | 17.76±1.35* | 16.45±1.54* |
| | 21 | 17.89±1.46* | 18.23±1.47* | 17.95±1.44* | 20.05±1.85* | 20.24±1.95* |
| | 7 | 14.56±0.92* | 11.56±0.98* | 12.45±1.17* | 15.38±1.45* | 14.67±1.36* |
| 7.5 | 14 | 25.43±2.42* | 24.83±2.35* | 22.08±2.11* | 25.84±2.96$^{NS}$ | 26.56±1.63* |
| | 21 | 29.28±3.04$^{NS}$ | 29.97±2.81* | 30.45±2.64* | 34.79±3.40* | 36.56±3.48* |
| | 7 | 17.35±1.20* | 16.87±0.78* | 16.89±1.52* | 18.12±1.15* | 18.73±1.30* |
| 10 | 14 | 31.18±2.71* | 29.78±1.79* | 27.72±2.50* | 31.45±3.05* | 32.74±3.71$^{NS}$ |
| | 21 | 38.65±3.48* | 36.28±4.02$^{NS}$ | 39.95±3.58* | 39.56±3.77* | 42.35±3.75* |

* p > 0.05, significance compared to oil samples without any added TRF at 0 day storage
NS: Non Significant

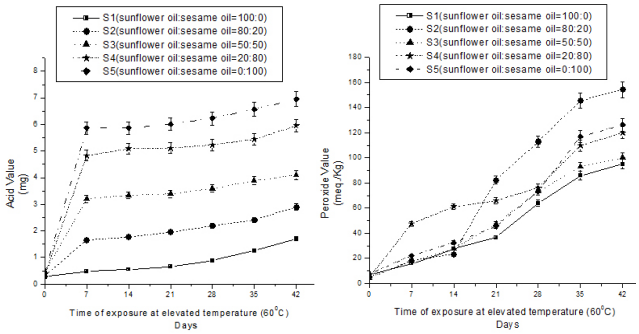


Figure 1(a). Graphical representation of effect of exposure at elevated temperature (60°C) on acid value of the sunflower, sesame oil and their blends

Figure 1(b). Graphical representation of effect of exposure at elevated temperature (60°C) on peroxide value of the sunflower, sesame oil and their blends

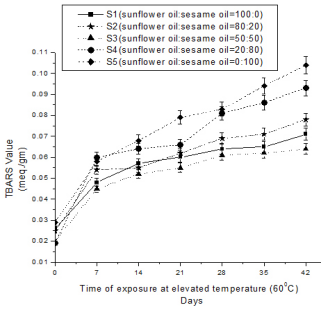


Figure 1(c). Graphical representation of effect of exposure at elevated temperature (60°C) on TBARS value of the sunflower, sesame oil and their blends

the formation of an unstable chemical structure in the blends. The final peroxide values obtained after 42 days of storage for S1, S2, S3, S4 and S5 were observed to be 94.89, 154.12, 100.1, 119.95 and 126.32 meq/Kg, respectively. Hence, the decreasing order of storage stability of these oil samples on the basis of PV can be written as S1>S3>S4>S5>S2.

Figure 1 (c) shows the effect of exposure at elevated temperature (60°C) on TBARS value of the oil samples. Initially, the respective TBARS value for the samples S1, S2, S3, S4 and S5 were found to be 0.026, 0.019, 0.020, 0.025 and 0.029 meq/g, respectively, whereas, the final TBARS values obtained for the respective samples after 42 days of exposure to 60°C were measured to be 0.071, 0.093, 0.064, 0.078 and 0.104 meq/g. In each oil sample, TBARS values increased gradually with respect to time of exposure during storage at 60°C due to accumulation of secondary oxidation products mainly malonaldehyde. The increase was the highest in case of S5, while S3 showed the least increase in TBARS value upon storage. A steeper slope can be seen initially in each oil sample due to higher rate of production of malonaldehyde. After that, the slopes became less steep with the progress of time of exposure except S4 and S5. In case of S4 and S5, the samples with higher proportions of sesame oil, it has been found that the rate of increase in TBARS value is the maximum, as was observed in case of acid value. The decreasing order of storage stability of these oil samples on the basis of TBARS value was S3>S1>S2>S4>S5.

*Effect of exposure at elevated temperature (60°c) on sunflower, sesame oil and their blends with added TRF*

Figure 2 (a) describes the effect of exposure at elevated temperature (60°C) on acid value of the oil samples with added TRF. The initial acid values for S1′, S2′, S3′, S4′ and S5′ were observed to be 0.17, 0.30, 0.47, 0.35 and 0.43 mg on 0 day respectively. The AV of each sample has been found to increase with the increase in time of exposure due to decomposition of oil glycerides into free fatty acids. Initially the slopes of the curves for S2′, S3′, S4′ and S5′ became high when a drastic increase in AV was observed from 0.3, 0.47, 0.35 and 0.43 mg on 0 day to 3.03, 1.48, 4.32 and 5.61 mg on 7$^{th}$ day for S2′, S3′, S4′ and S5′, respectively possibly due to the production of free fatty acids at a higher rate. The curve obtained for S1′ was found to be almost parallel to the time of exposure axis which may indicate that the AV for S1′ has been increased at a very low rate. The final acid values obtained for S1′, S2′, S3′, S4′ and S5′ after

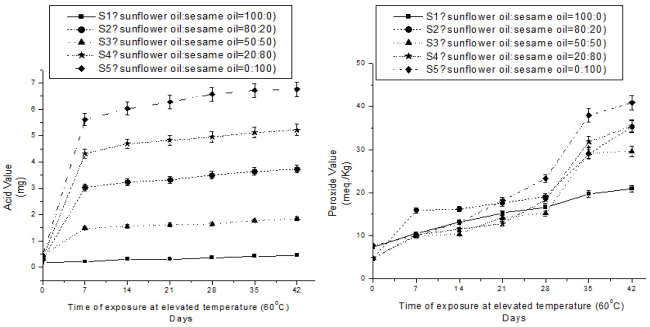


Figure 2(a). Graphical representation of effect of exposure at elevated temperature (60°C) on acid value of the sunflower oil, sesame oil and their blends with added TRF

Figure 2(b). Graphical representation of effect of exposure at elevated temperature (60°C) on peroxide value of the sunflower oil, sesame oil and their blends with added TRF

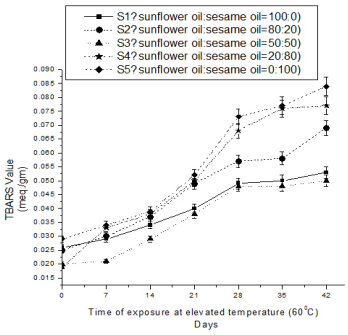


Figure 2(c). Graphical representation of effect of exposure at elevated temperature (60°C) on TBARS value of the sunflower oil, sesame oil and their blends with added TRF

42 days of storage were measured to be 0.45, 3.73, 1.84, 5.22 and 6.76 mg, respectively. In this study, the sample S1′ was found to be the most stable oil followed by S3′, S2′, S4′ and S5′ in terms of acid value.

Figure 2 (b) shows the effect of exposure at elevated temperature (60°C) on peroxide value of the oil samples with added TRF. Initially the peroxide values were observed to be 7.23, 4.69, 4.73, 4.81 and 7.76 meq/Kg on 0 day for S1′, S2′, S3′, S4′ and S5′, respectively. The PV for each oil sample has been found to increase with the increase in time of exposure at 60°C. The steepness of the curve for S2′ was slightly more as compared to other samples initially when an increase from 4.69 meq/Kg on 0 day to 15.91 meq/Kg on 7$^{th}$ day was observed. Eventually, the rate of increase in PV for each sample has been noticed to be low possibly due to their tendency to reach the state of equilibrium between the rate of formation of hydroperoxides and their rate of decomposition. Although a drastic increase in PV was noticed for the samples S2′, S3′, S4′ and S5′ when the values were increased from 19.09, 15.22, 18.31 and 23.27 meq/

Kg on 28th day to 29.00, 29.23, 31.83 and 37.97meq/Kg on 35th day for the respective samples. It may indicate that the formation rate of hydroperoxides outweigh their rate of decomposition. However, S1′ showed the least increase in PV during the storage study. Finally after 42 days of storage, the respective peroxide values for S1′, S2′, S3′, S4′ and S5′ were observed to be 20.89, 35.27, 29.61, 35.51 and 45.90 meq/Kg. The decreasing order of storage stability on the basis of PV for these oil samples was found to be S1′>S3′>S2′>S4′>S5′, as was observed in case of AV.

Figure 2 (c) describes the effect of exposure at elevated temperature (60°C) on TBARS value of the oil samples with added TRF. The initial TBARS values for S1′, S2′, S3′, S4′ and S5′ were measured to be 0.026, 0.025, 0.02, 0.019 and 0.029 meq/g. In this figure, it was clearly seen that the slope of the curve for each oil sample has not been found to show any drastic increase in TBARS value throughout the storage period possibly due to the action of TRF which may retard the formation of secondary oxidation products mainly malondialdehyde. The final TBARS values after 42 days of storage were observed to be 0.053, 0.069, 0.05, 0.077 and 0.084 meq/g for S1′, S2′, S3′, S4′ and S5′, respectively. The order of the storage stability for these oil samples in terms of TBARS value was found to be S3′>S1′>S2′>S4′>S5′, as was observed in case of oil samples without TRF under the same condition of storage.

*Comparative study between the blends of sunflower oil and sesame oil (50:50) with and without added TRF*

Initially on 0 day, the acid value of 0.47 mg was observed for both S3 and S3′ and finally on 42nd day, the respective values for S3 and S3′ were measured to be 4.10 mg and 1.84 mg for S3 and S3′. The sample S3′ has been found to show less increase in AV with respect to time of exposure possibly due to the activity of TRF which may reduce oil deterioration by hydrolysis to a great extent, while increase in free fatty acid development in S3 has been very high throughout the storage period. The sample S3′ showed better storage stability as compared to that of S3 under the same storage condition in terms of AV.

Initially on 0 day, a peroxide value of 4.73 meq/Kg was measured for both the samples. As the time of exposure increases, the PV of each sample has been found to increase. This increase was more in case of S3, while S3′ showed the minimal increase in PV with the progress of time of exposure may be due to quenching activity of TRF for free radicals produced during auto-oxidation. Finally, after 42 days of storage, peroxide values of the samples S3 and S3′ have been measured to be 100.1 meq./Kg and 29.61 meq./Kg, respectively. From the view point of PV, S3′ has been found to show much better storage stability than that of S3 under the same condition of storage.

Initially, the same TBARS value of 0.02 meq/g on 0 day was measured for both the samples. The TBARS value of the samples has been found to increase with the progress of time of exposure, but in case of S3′, the increase in TBARS value was observed at a comparatively lower rate possibly due to the scavenging activity of TRF to reduce the degradation of hydroperoxides into secondary oxidation products mainly malondialdehyde during auto-oxidation of oil. The TBARS values for S3 and S3′ on 42nd day of storage were found to be 0.064 and 0.05meq/g respectively. The sample S3′ showed the better stability under storage as compared to that of S3 at the same condition of storage on the basis of TBARS value.

## Discussion

The blended sample S3 (sunflower oil: sesame oil = 50:50) showed minimum increase in TBARS value upon exposure at 60°C at the end of 42 days of storage. But in case of AV and PV, S1 showed the minimum increase and S3 sample exhibited values higher than that of S1. It is well known that sesame oil possesses many nutritional as well as medicinal properties like anti-cancer activity, prevention of thrombosis, reduction in blood pressure and cardiac hypertrophy, cholesterol lowering effect in human body by lowering the absorption of cholesterol and fatty acids in lymph and protective activity for LDL from oxidative modifications by slowing down the progression of atherogenesis. So, considering the beneficial effects of sesame oil over pure sunflower oil (S1), the blend S3 was selected as the best among all the oil samples. Attempt has been done to further improve the S3 blend by addition of natural antioxidant rich extract.

Among the samples, the blend S3′ (sunflower oil:sesame oil = 50:50) has been found to exhibit the highest stability on the basis of TBARS value as the increase in TBARS value for this sample was minimum upon storage at 60°C for 42 days. However, AV and PV for S3′ have been observed slight higher as compared to those for the pure sunflower oil (S1′) which showed the minimum increase in AV and PV upon storage at the same condition. The combination of sunflower, sesame oil and TRF provides better

health benefits to humans in terms of medicinal and nutritional aspects like anticancer effect, cholesterol lowering effect, prevention of thrombosis, reduction in blood pressure, anti-carcinogenic activity and antioxidant effect.

## Conclusion

From the present study, the addition of tocotrienol rich fraction (TRF), used as a source of natural antioxidant, has been found to be effective on the improvement of oil stability at the optimal dose of 5 µl/g for sunflower, sesame oil and their blends. Oils containing TRF have been found to show better stability based on acid value (AV), peroxide value (PV) and thiobarbituric acid reactive substances (TBARS) value than those observed for oil samples without TRF under the same conditions of accelerated oxidation. Therefore, considering the combined beneficial effects of sunflower, sesame oil and TRF over the pure sunflower oil, the blend, containing sunflower and sesame oil in the volumetric ratio of 50:50 with TRF added in the concentration of 5 µl/g, has been thought to be the best among all the samples.

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