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Food Chemistry 105 (2007) 1076–1085
Food Chemistry
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Sesame lignans enhance the thermal stability of edible vegetable oils

S. Hemalatha, Ghafoorunissa *

Biochemistry Division, National Institute of Nutrition, Indian Council of Medical Research, Jamai Osmania P.O., Hyderabad 500 007, Andhra Pradesh, India

Received 27 November 2006; received in revised form 3 March 2007; accepted 9 May 2007

Abstract

The effect of sesame lignans on the thermal and storage stability of edible vegetable oils (soybean-SBO, sunflower-SFO and ricebran-RBO) was studied by (i) determining the total free radical scavenging activity (RSA) using DPPH, (ii) % total tocol retention, (iii) lignan profile and (iv) PUFA composition. The order of RSA and retention of total tocols of oils heated up to 120 min at frying temperature (FT) were RBO = SBO > SFO and RBO > SBO > SFO, respectively. Heating SBO or SFO at FT after addition of 1.2% lignans increased RSA of SBO to a greater extent than that of SFO, and increased retention of total tocols only in SBO. However, addition of lignans did not further increase the RSA of RBO. Heating oils with added lignans, increased sesamol and decreased sesamolin while sesamin was relatively resistant to heat. These findings suggest that sesame lignans may have potential application as natural antioxidants in the edible oil and food industry.

Keywords: DPPH; Edible vegetable oils; Radical scavenging activity; Sesamin; Sesamolin; Sesame lignans; Thermal stability; Storage stability; Total lignans; Total tocols

1. Introduction

The stability of oils during storage or upon heating to frying temperature (FT) is an important measure for ensuring good oil performance at elevated temperature. Oxidation of unsaturated fatty acids is one of the major causes of the development of off-flavor compounds and in reducing the nutritive value of food products. Antioxidants in foods play a major role in maintaining the quality of oils and foods. Usually, the native tocopherols that are normally present in the oils are sufficient to protect the oil against oxidative deterioration during storage at ambient temperature. However, higher temperature treatments like deep fat frying, require the use of more efficient antioxidants to delay the thermo-oxidative degradation of the oil. Hence, during refining of edible oils and in manufacture of ready to eat and processed foods, synthetic antioxidants are added at permissible levels (Awasthi, 2000). Recent studies have shown that synthetic antioxidants are not sufficiently effective in retarding deterioration of oils during frying (Kaitaranta, 1992). The current concern is regarding the possible adverse effects of synthetic antioxidants obtained from several components of diets (edible oils, processed and ready to eat foods) on health (Barlow, 1990; Ito, Hagiwara, Shibata, Ogiso, & Fukushima, 1982). It is therefore important to identify novel natural components of foods having radical scavenging or antioxidant activity (Shahidi, 1997). Sesame contains substantial amounts of unique components, namely lignans (sesamin and sesamolin), which contribute to the inherent higher stability of sesame oil (Budowski, 1950). Our earlier studies have shown that sesame lignans inhibit oxidative damage in *in vitro* biological systems (Ghafoorunissa, Hemalatha, & Vishnuvardhana Rao,

Abbreviations: DPPH, 2,2-diphenyl picryl hydrazyl; RBO, rice bran oil; RSA, radical scavenging activity; SBO, soybean oil; SFO, sunflower oil; TL, total lignans; TT, total tocols.

* Corresponding author. Address: H.No 10-2-289/37, Flat #402, Maphar Comfortek Apartments, Shantinagar Colony, Masabtank, Hyderabad 500 028, Andhra Pradesh, India. Tel.: +91 04 23324138.
E-mail addresses: s_hema999@yahoo.co.in (S. Hemalatha), ghafoorunissanin@yahoo.com (Ghafoorunissa).

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doi:10.1016/j.foodchem.2007.05.023

2004). Sesame cake extract has been shown to offer protection to vegetable oils (Suja, John, Selvam, Jayalekshmy, & Armugham, 2004). In the present study the effectiveness of sesame lignans (crystallized sesamin and sesamolin) were evaluated on the thermal and storage stability of edible vegetable oils by determining the total RSA, total tocol (TT) retention, lignan profile and PUFA composition.

2. Materials and methods

2.1. Chemicals and reagents

Sesamol and tocopherols (α, γ and δ) and 2,2 diphenyl-1-picryl hydrazyl (DPPH) were procured from Sigma Chemical Co. (St. Louis, MO, USA), and α-tocotrienol from R. Hoffman–La Roche & Co. Ltd. Basel, Switzerland. Methanol, acetonitrile, *n*-hexane and ethyl acetate (HPLC grade) were procured from Qualigens India Ltd. All other chemicals and reagents used were of analytical grade procured from local sources. Refined soybean (SBO), sunflower (SFO) without added antioxidants were kindly donated by Ruchi Health Foods Ltd., Kannigaipair Village, Thiruvallur Dist., Tamilnadu, India. Rice-bran oil (RBO) was kindly donated by SSD Oil Mills Company Ltd., 132, Village Road, Iyyappanthangal, Chennai, India.

2.2. Isolation and crystallization of sesamin and sesamolin

Sesamin and sesamolin were isolated and crystallized from sesame oil (Indian sesame cultivars having medium lignan content) as described in our earlier publication (Hemalatha & Ghafoorunissa, 2004). Briefly, sesame oil (500 g) was dissolved in acetone (1:8 v/v) and cooled overnight at −70 °C. The glyceride crystals were separated by filtration, while the mixture was kept at −20 °C. Removal of acetone from the filtrate gave a yellow oil which when mixed with *iso*-octane and left at 4 °C for 4–5 days yielded sesamin crystals. The filtrate, after evaporation of *iso*-octane, was saponified with ethanolic potassium hydroxide and the mixture diluted with water and extracted with ether. The filtrate, after removal of ether, was dissolved in chloroform and petroleum ether. Sesamolin crystallized from the solution. Sesamin was recrystallized in ethanol while sesamolin was recrystallized using chloroform petroleum ether mixture. The purity of sesamin and sesamolin obtained, was above 90%.

2.3. Selection of oils

The selection of test oil was based on the presence of varying PUFA composition. (a) Oil having high linoleic acid (18:2*n* − 6), SFO; (b) oil having alpha-linolenic acid (18:3*n* − 3) in addition to linoleic acid, SBO; and (c) oil having moderate 18:2*n* − 6, RBO.

2.4. Addition of purified lignans (sesamin and sesamolin) to test oils

The stability of the above oils with or without added lignans was assessed both at room temperature (RT) and by heating the oil to FT. Both sesamin and sesamolin (crystals) are lipid soluble compounds. Sesamin (1.6 or 3.2 g) and sesamolin (0.8 or 1.6 g) were added to 20–25 ml of each of the test oils and slightly warmed to dissolve the purified lignans. After cooling, the test oils with added lignans were made up to 200 ml with the respective oil.

After addition of sesamin and sesamolin to each of the test oil, an aliquot of 10 ml of each test of the oil with or without added lignans was stored in brown bottles and kept at RT for 60 days to assess the storage stability. The remaining (190 ml) test oil was preheated to FT (180 °C).

2.5. Preparation of puries

Puries are food products prepared from wheat dough. To 600 g of whole wheat flour, 400 ml of water was added and mixed to obtain dough. Puries are circular in shape and were prepared by spreading 8–10 g of the dough to uniform thickness and size and were deep fried in oils at FT. The FT was monitored throughout the frying period using a thermometer. The FT of the oil was maintained by frying puries for 120 min.

2.6. Assessment of thermal and storage stability

Aliquots of the storage oil at 15 days intervals (0, 15, 30 and 60 days) and heated oils at 30 min intervals (0 min, 30 min, 60 min, 90 min and 120 min) were assessed for their stability, by determining total RSA of oils using DPPH, total tocol content, lignan profile and PUFA composition.

2.7. DPPH assay

DPPH assay is a widely used method used to study the antioxidant capacities of natural products, individual antioxidants and vegetable oils (Espin, Soler-Rivas, & Wichers, 2000). DPPH is a stable free radical and has a dark violet colour. It has maximum absorption at 517 nm, and the absorption of the DPPH radical is decreased in the presence of an antioxidant. The dose dependent effect of oils with or without (0.6% and 1.2%) added lignans was studied on RSA of DPPH. Briefly, the oil samples ranging between 0–80 mg of SBO or RBO and 0–300 mg of SFO were made up to 3 ml with ethyl acetate and 1 ml of 1 mM DPPH (in ethyl acetate) was added. After 10 min, when the absorbance reached a constant value, the decrease in the absorbance was determined at 517 nm. The control was the DPPH solution without vegetable oil and blank was ethyl acetate. 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 concentration of the oil sample (milligrams) and IC_{50} value was calculated. IC_{50} is defined as the amount of original oil sample in milligrams required to reduce the initial DPPH concentration by 50% (as was extrapolated from the dose response curve) divided by the total volume of DPPH solution with added solution of the oil. As comparison α-tocopherol was evaluated under the same experimental conditions.

2.8. Analysis of lignan and tocopherol profile

The lignan and tocopherol profile was determined (Barrie & Linda, 1989; Kikugawa, Arai, & Kurechi, 1983) by direct oil injections (1:100 w/v, oil and *n*-hexane for lignan profile, 1:20 w/v, oil and *n*-hexane for tocol profile) using a Shimadzu HPLC Class VP Multisystem (Asian Pacific PTE

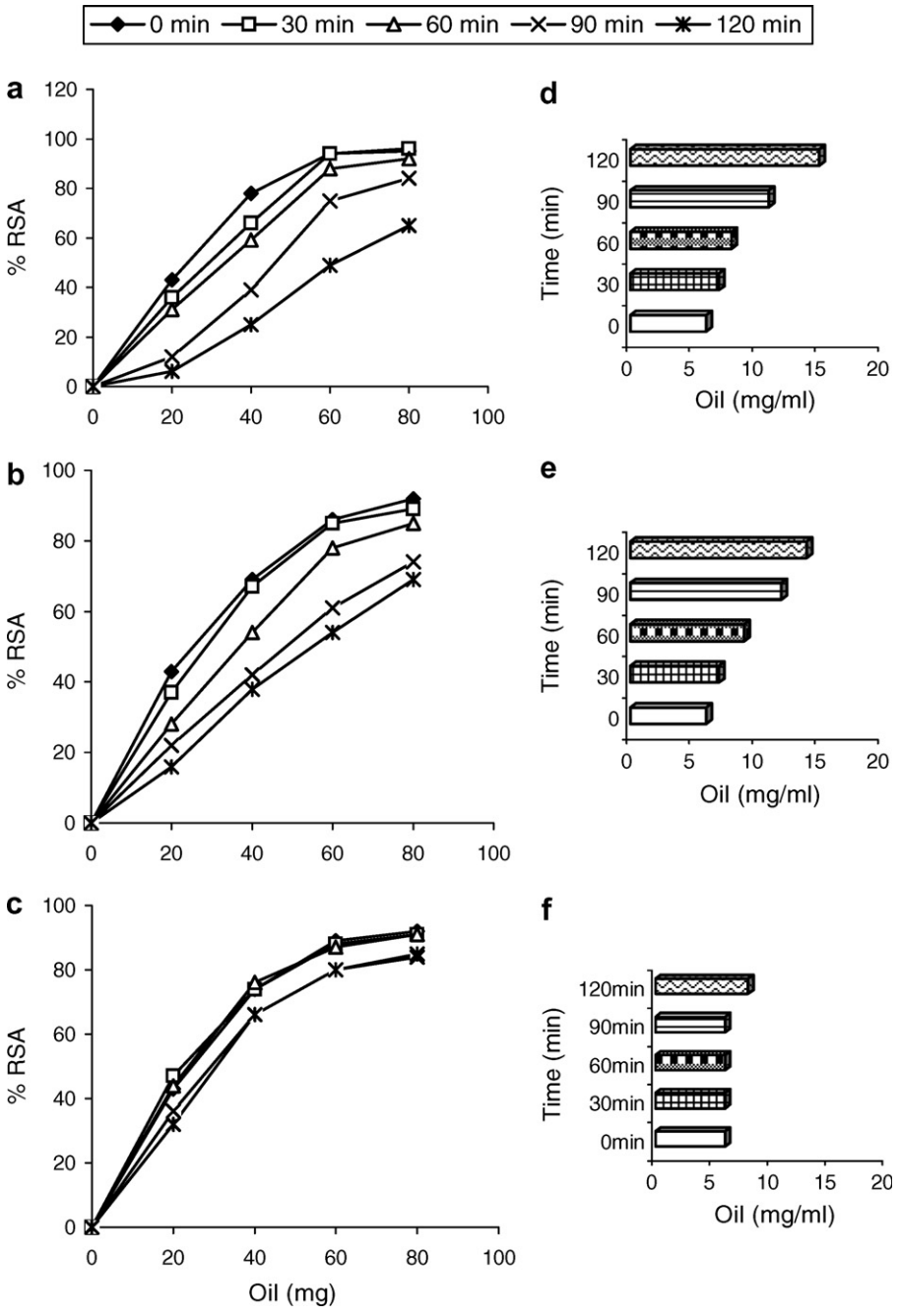

Fig. 1. Inhibitory effects of SBO with or without sesame lignans on DPPH radical: (a) SBO; (b) SBO + 0.6% lignans; (c) SBO + 1.2% lignans. Insets (d)–(f) shows the IC_{50} values of each oil at different time points. Legends for insets (d)–(f): □ 0 min; ▦ 30 min; ▩ 60 min; ▤ 90 min; and ▨ 120 min.

Ltd., Japan). The system was equipped with a Shodex C18 column (4.6 mm i.d. × 250 mm), connected to SIL-10AD$_{VP}$ auto injector and a Shimadzu SPD-10AV$_{VP}$ UV–VIS detector. The peaks were identified and quantitated using Shimadzu CLASS VP V6.14 SP1 version in comparison with those of authentic lignans (sesamol procured from sigma, sesamin and sesamolin isolated and crystallized in our laboratory) and tocols (α-, γ-, δ-tocopherol and α-tocotrienol) used as external standards. Sesamol or α-tocopherol was used as internal standards for calculating the % recovery of lignans and tocols. Assays ($n = 2$) were performed with freshly prepared solutions in duplicate.

2.9. Analysis of PUFA

The oil samples were transmethylated using a solution of 2% H_2SO_4 in methanol. The fatty acid methyl esters were analyzed by Gas Chromatography (Ghafoorunissa, Reddy, & Sesikaran, 1995) by Perkin Elmer gas

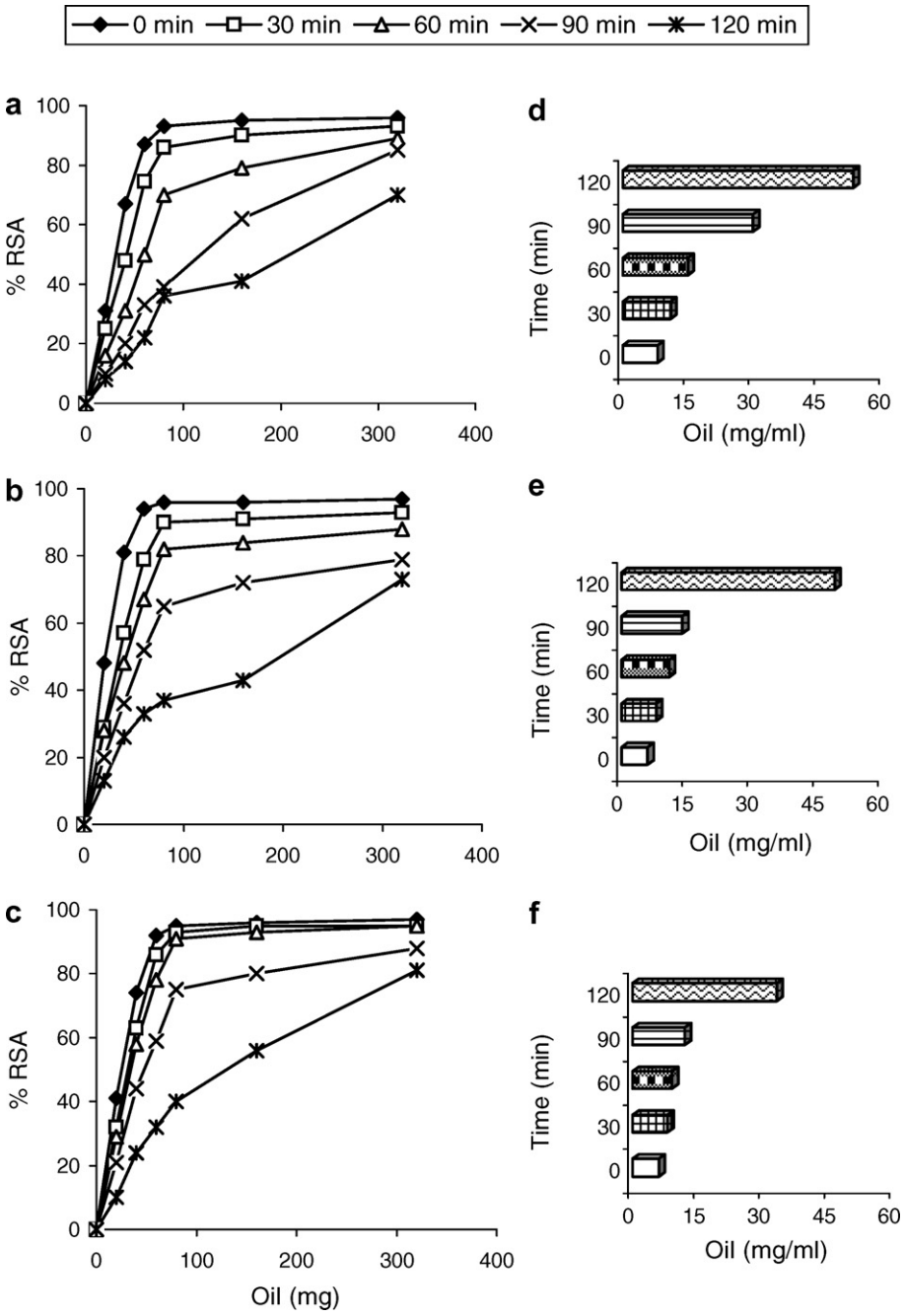

Fig. 2. Inhibitory effects of SFO with or without sesame lignans on DPPH radical: (a) SFO; (b) SFO + 0.6% lignans; (c) SBO + 1.2% lignans. Insets (d)–(f) shows the IC_{50} values of each oil at different time points. Legends for insets (d)–(f): □ 0 min; ▦ 30 min; ▩ 60 min; ▤ 90 min; and ▨ 120 min.

chromatograph (Norwalk, Conn) using SP-2330 capillary column (30 m × 0.32 mm i.d. Supelco, USA). The packing material constitutes bis-cyanopropyl and cyanopropylphenyl siloxane. Individual fatty acids were identified using authentic standards (Nu-Chek, Elysian, Minn). Heptadecanoic acid was used as an internal standard.

For all the above analysis, two independent assays ($n = 2$) were performed, each in duplicate. Since only two independent assays were carried out, the results are expressed as a mean of the two assays.

3. Results and discussion

3.1. General

The non-glyceride fraction of oils has variable composition of tocols and unique components that not only contribute to the oxidative stability of edible vegetable oils but also provide dietary antioxidants (Shahidi & Shukla, 1996). Domestic or industrial applications with higher temperature treatments, require the use of more efficient

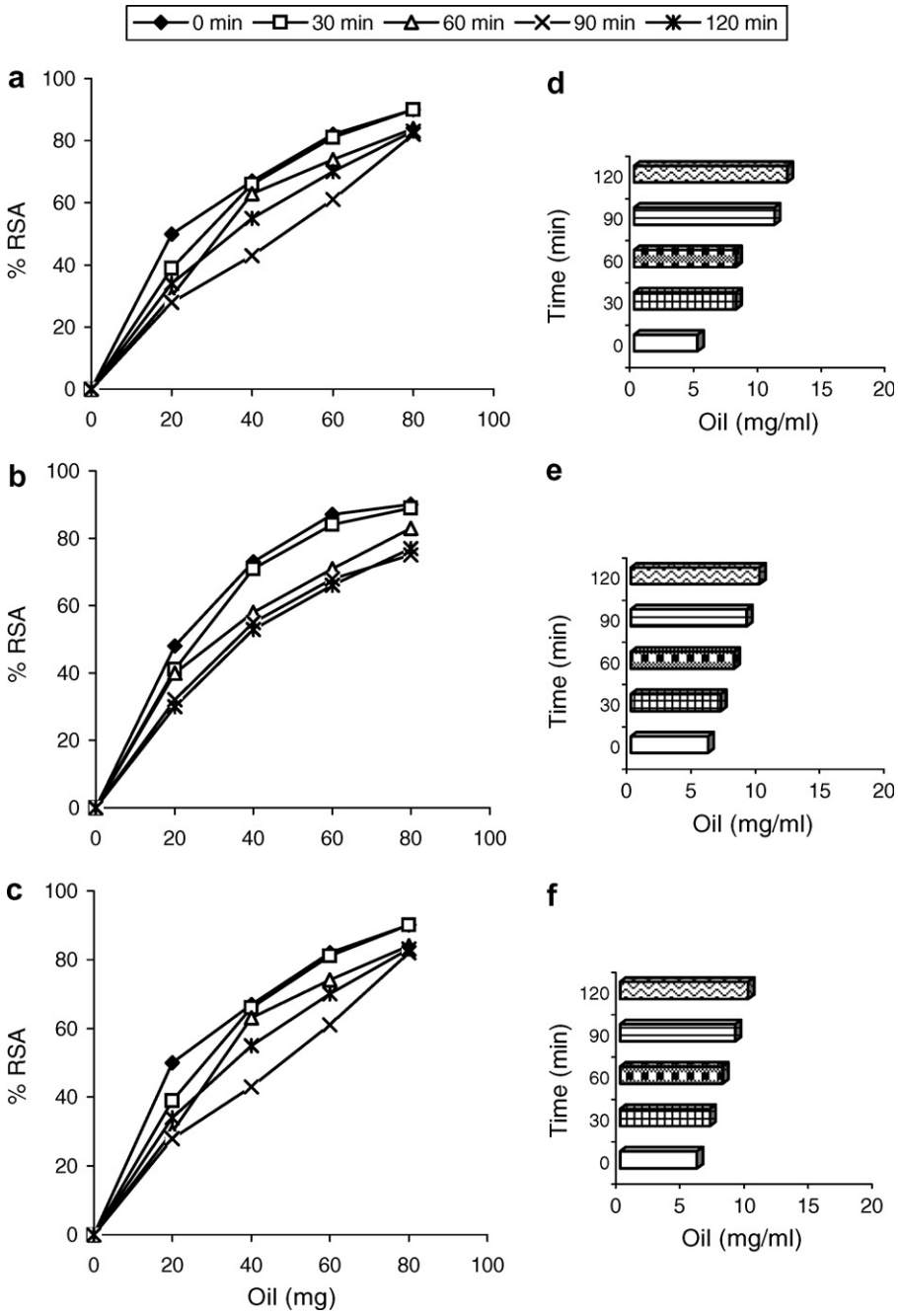

Fig. 3. Inhibitory effects of RBO with or without sesame lignans on DPPH radical: (a) RBO; (b) RBO + 0.6% lignans; (c) RBO + 1.2% lignans. Insets (d)–(f) shows the IC_{50} values of each oil at different time points. Legends for insets (d)–(f): □ 0 min; ▦ 30 min; ▩ 60 min; ▤ 90 min; and ▨ 120 min.

antioxidants to delay the oxidative degradation of the oil. Sesame is an important source of edible oil and has also been used as a health food in India for its nutritional and medicinal value. The endogenous lignans (sesamin and sesamolin) along with γ-tocopherol confer superior oxidative stability to sesame oil as compared to other vegetable oils. Sesamol is formed from sesamolin due to chemical transformation during processing conditions (Fakuda, Nagata, Osawa, & Namiki, 1986). The present work demonstrates the effect of sesame lignans on thermal or storage stability of edible vegetable oils with varying PUFA composition.

3.2. Thermal stability of oils

3.2.1. Total radical scavenging activity by DPPH assay

All the oils used in this study were devoid of the synthetic antioxidants (that are added during industrial processing of edible oils). The total PUFA content of oils used in this study was as follows: SBO 61%, SFO 58% and RBO 34%. The oils contained unique minor components such as oryzanol and tocotrienols in RBO; isoflavones and tocopherols (α, γ and δ) in SBO; and α-tocopherol in SFO. The IC_{50} values, before heating the oils to FT, was essentially similar (SBO – 6 mg; SFO – 8 mg; RBO – 5 mg/ml) in all the oils (Figs. 1d, 2d and 3d). Heating oils with or without addition of lignans at FT resulted in a time dependent decrease in the RSA (Figs. 1d, 2d and 3d). The relative order of the RSA of oils after heating the oils to FT (Figs. 1d, 2d and 3d) was as follows: RBO = SBO > SFO (IC_{50}: RBO – 11 mg, SBO – 15 mg and SFO – 53 mg). In SBO (Fig. 1d) and RBO (Fig. 3d) the RSA was comparable between 0 min and 60 min of heating time and decreased after 90 min at FT (Figs. 1d and 3d). In SFO (Fig. 2d) the RSA was comparable between 0 min and 30 min of heating time and decreased after 60 min at FT. The RSA of SFO or SBO before heating and that of SBO after heating for 120 min at FT was higher than that reported (Valavanidis et al., 2004). However, the RSA of SFO after heating the oil for 120 min was lower than that documented (Valavanidis et al., 2004). The higher RSA of SBO compared to SFO (with similar PUFA content) is in agreement with other reports in literature (Espin et al., 2000; Kalantzakis, Blekas, Pegklidou, & Boskou, 2006; Valavanidis et al., 2004). The higher RSA of RBO (available in Indian market) may be attributed to its lower PUFA levels as compared to SBO and SFO and to its unique minor components (γ-oryzanol and tocotrienols). Published reports have documented that γ-oryzanol has high antioxidant activity against cholesterol oxidation when compared to α or γ tocopherols or tocotrienols (Xu, Hua, & Godber, 2001), and has higher DPPH radical scavenging activity and improved oxidative stability when added to pharmaceutical oils (Juliano, Cossu, Alamanni, & Piu, 2005) and food (Nanua, McGregor, & Godber, 2000).

Compared to SFO alone (Fig. 2d), addition of 0.6% or 1.2% lignans to SFO increased the RSA of SFO by 53% (Fig. 2e) and 60% (Fig. 2f), respectively at the end of 90 min of heating (IC_{50}: SFO: 30 mg oil; SFO + 0.6% lignans: 14 mg oil; SFO + 1.2% lignans: 12 mg/ml). Addition of 1.2% lignans to SFO increased RSA (by 40%) at the end of 120 min of heating at FT (IC_{50}: SFO: 53 mg oil; SFO + 1.2% lignans: 33 mg oil). These results indicate that addition of 0.6% lignans to SFO may confer protection for up to 90 min of heating at FT, while addition of 1.2% lignans has shown protection for up to 120 min at FT. Previous reports have shown that it was very difficult to improve the oxidative stability of SFO, either by adding natural or synthetic antioxidants (Yaneshlieva & Marinova, 2001). With addition of 1.2% lignans to SBO, the RSA was comparable between 0 and 120 min of heating at FT (Fig. 1f). Addition of 1.2% lignans to SBO (Fig. 1f) increased RSA (by 47%) at the end of 120 min of heating at FT as compared to SBO (Fig. 1d) alone (IC_{50}: SBO: 15 mg oil; SBO + 1.2% lignans: 8 mg oil). The addition of lignans to RBO did not further increase RSA. The relative order of RSA of oils with added

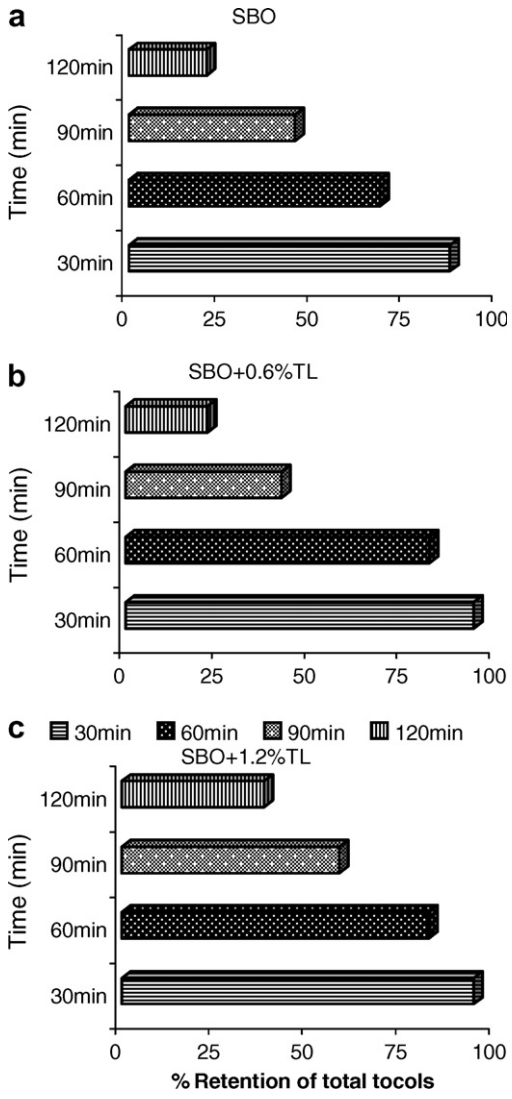

Fig. 4. Percent retention of total tocols in SBO with or without lignans heated at FT: (a) SBO; (b) SBO + 0.6% TL; and (c) SBO + 1.2% TL.

lignans after heating to FT for 120 min was as follows: RBO = SBO > SFO. Chu (1991) has demonstrated that a blend of SBO and sesame oil has the longest frying life and most stable amongst all the oils. Suja et al. (2004) have shown that sesame cake extract offers protection to vegetable oils. This study demonstrates the higher RSA of edible vegetable oils with added lignans (sesamin and sesamolin) possibly due to synergism between sesame lignans and the non-glyceride components of SBO (soya lignans, isoflavanoids) or SFO (phytosterols).

3.2.2. Total tocols (TT)

The % retention of TT of oils is presented in Figs. 4–6. Heating oils with or without addition of lignans at FT, resulted in a time dependent decrease in TT content (Figs. 4–6). The relative order of the retention of TT of oils heated up to 120 min at FT was as follows: RBO > SBO > SFO. The retention of TT was higher, between 30 and 90 min (87–68%) in SBO (Fig. 4a) as compared to SFO (Fig. 5a). In SFO there was about 54% reduction at the end of 30 min of heating time and decreased to 25% at 90 min (Fig. 5a) and this was comparable to the degradation of TT in SBO at 120 min (Fig. 4a). The increased retention of TT in SBO may be attributed to its high δ-tocopherol content as studies have shown that α-tocopherol is least resistant to temperature followed by β- and γ-tocopherol, while the highest thermal stability was shown by δ-tocopherol (Gordon & Kourimska, 1995). Addition of 1.2% lignans to SBO increased retention of TT at the end of 120 min of heating (Fig. 4c) as compared to SBO (Fig. 4a). Chung, Lee, and Choe (2004) have demonstrated that tocopherol stability during heating was higher in SBO, sesame oil blend. The findings of the present study are in agreement with that which has been reported in literature (Chung et al., 2004). The retention of TT was similar with or without the addition of (0.6% or 1.2%) lignans at all time points (30–120 min) of heating in both SFO and

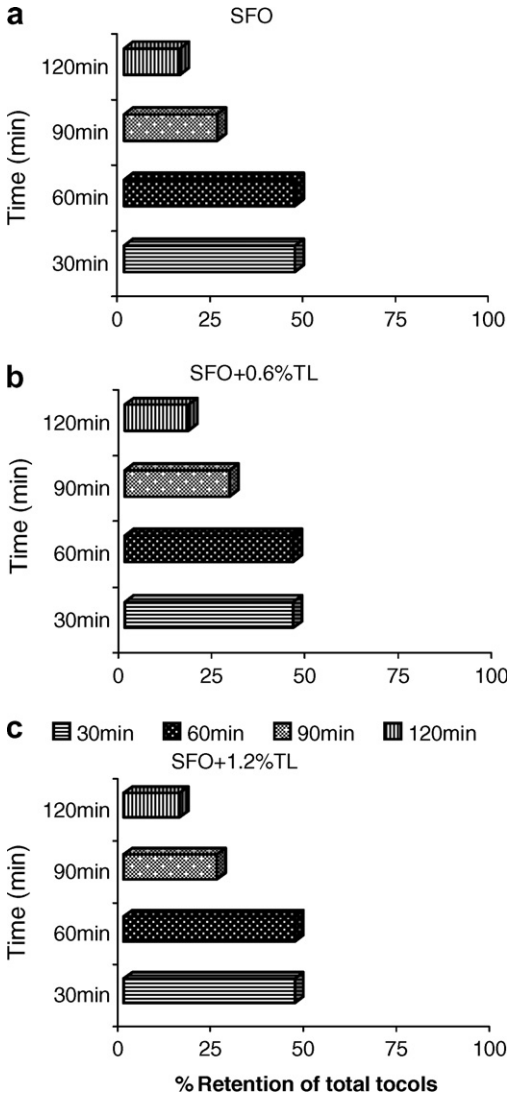

Fig. 5. Percent retention of total tocols in SFO with or without lignans heated at FT: (a) SFO; (b) SFO + 0.6% TL; and (c) SFO + 1.2% TL.

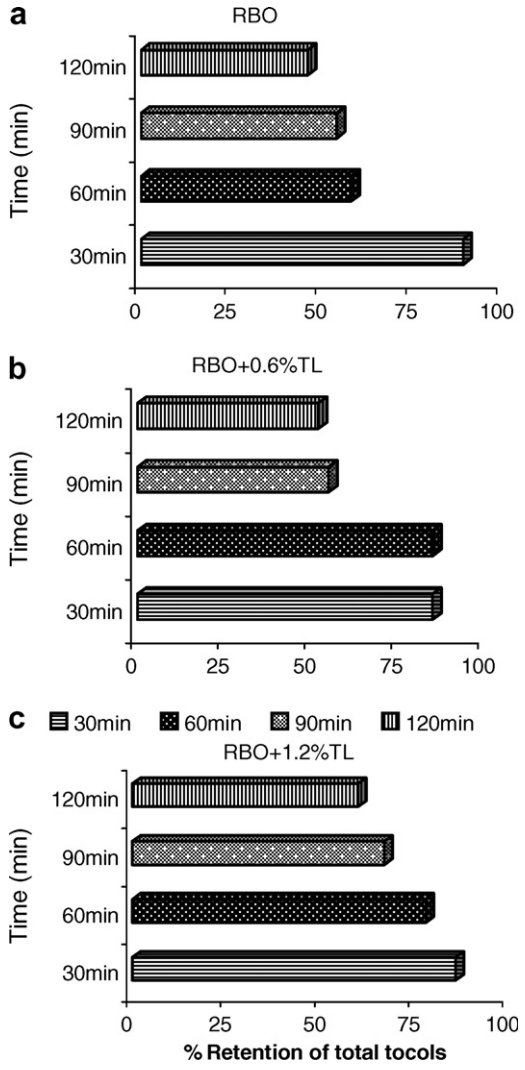

Fig. 6. Percent retention of total tocols in RBO with or without lignans heated at FT: (a) RBO; (b) RBO + 0.6% TL; and (c) RBO + 1.2% TL.

RBO (Figs. 5a–c and 6a–c). In our earlier studies, higher synergistic antioxidant effects were observed with sesame lignans and tocotrienols as compared to lignans and tocopherols in *in vitro* lipid peroxidation systems (Ghafoorunissa et al., 2004). However, these synergistic effects were not observed in RBO with added sesame lignans despite the fact that RBO contains both tocopherols and tocotrienols. The lack of synergistic effect of sesame lignans with RBO could possibly be attributed to the lower levels of TT (~350 mg/kg oil) present in RBO. These synergistic effects of lignans with tocols, have nutritional and therapeutic implications. Therefore, consuming foods containing sesame seeds or sesame oil may prove beneficial for enhancement of vitamin E status and health.

3.2.3. Total lignans (TL)

The lignan profile of oils heated at FT is shown in Fig. 7. Heating oils at FT resulted in a decrease in the TL content, possibly due to the decomposition of sesamolin (Fig. 7). This was perhaps due to the formation of sesamol from non-detectable levels in all the oils with added lignans (Fig. 7a–f). According to Fakuda et al. (1986) the decomposition of sesamolin was almost complete within 120 min. In the present study, 36–74% of sesamolin retained after heating oils (with added lignans) for 120 min (Fig. 7). Sesamol reached a maximum at 60 min and gradually decreased at 120 min of heating at FT. However, the percentage of sesamol formed during 60 min heating at FT, was between 0.04–0.06% in oils with 0.6% TL and 0.04–0.09% in oils with 1.2% TL. That sesamol concentration reached a maximum not exceeding 0.1% in the oil at FT following gradual reduction is in agreement with that reported in literature (Fakuda et al., 1986). Nasirullah and Baby Latha (2005) have shown that palmolein: sesame oil blend was more stable than sesame oil: SFO blend and there was equal distribution of sesamol in oil left over after deep frying and in the oil absorbed by the product. In this study, the

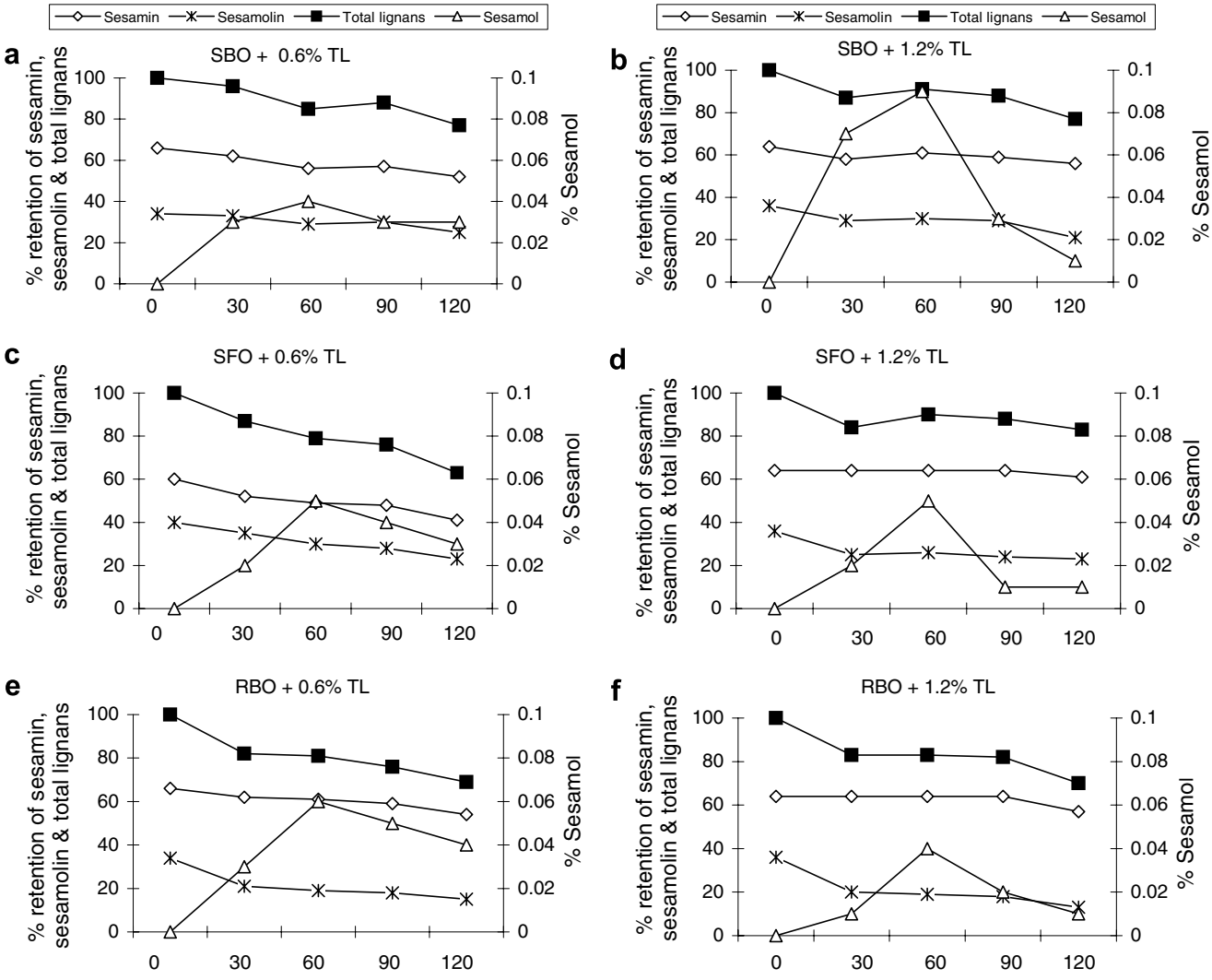

Fig. 7. Lignan profile of oils heated at FT.

retention of sesamin was between 70% and 90% at the end of 120 min at FT, suggesting that sesamin is more resistant to heat. Similarly, Chung et al. (2004) have also shown that sesamin content was relatively more stable in a SBO; sesame oil blend heated at 160 °C continuously for 25 h. Sesamin, the major lignan in sesame oil has been reported to exhibit multiple biological functions (Sugano & Akimoto, 1993). Sesamolin has been related to its high stability against oxidation and increased storage of sesame oil. Therefore, blending of vegetable oils with sesame oil may impart several nutritional and health benefits (Jeng & Hou, 2005) as compared to addition of synthetic antioxidants.

3.2.4. Total PUFA

At the end of 120 min of heating total PUFA decreased to the same extent (90%) in all the oils with or without the addition of lignans (data not shown).

3.3. Storage stability

In all the three oils, storage for 60 days at RT did not affect the RSA, retention of total tocols, total lignans and total PUFA (data not shown). Shahidi, Amarowicz, Abou-Gharbia, and Shehata (1997) have shown that the loss of sesamin, sesamolin and γ-tocopherol was higher after storage of sesame oil for 35 days at 65 °C. Shiela, Sreerama, and Gopala Krishna (2004) have shown that RBO, palmolein or sesame oil either individually or in combination with SFO, groundnut oil and mustard oil showed the same trend in the retention of minor components, upon storage for six months at RT. Yang, Chu, and Liu (2005) have demonstrated that SBO without any added antioxidant was of acceptable quality for 12 months of storage. Our results are in agreement with the reports in literature (Shiela et al., 2004; Yang et al., 2005).

In conclusion, the observed higher thermal stability of edible vegetable oils after addition of sesame lignans suggests that: (a) sesame lignans may have potential application as natural antioxidants in the edible oil and food industry; (b) blends of sesame oil with preferred oil (s) might increase the antioxidant potential of oils; and (c) therefore, blending of vegetable oils with sesame oil may be more effective than the addition of synthetic antioxidants. Apart from providing stability, lignans may also increase antioxidant potential of diets since its vitamin E sparing effect has been demonstrated both in *in vitro* and *in vivo* studies (Ghafoorunissa et al., 2004; Hemalatha, Raghunath, & Ghafoorunissa, 2004).

Acknowledgements

We thank the Ministry of Consumer Affairs, Food and Public Distribution (Department of Food and Public Distribution) Directorate of Vanaspathi, Vegetable Oils and Fats, New Delhi for providing financial support.

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