Journal of Food Technology 1 (3): 97-101, 2003

High Performance Liquid Chromatographic Determination of Seed D-form (*RRR*-) Tocopherol Homologues of Sesame (*Sesamum indicum* L.) Spreads: Process Effects on their Quantities

[1]Ö. Tokuşoğlu, [2]I. Durucasu , [2]F. Yemiş and [3]Z. Yildirim
[1]Celal Bayar University, Akhisar Food M.Y.O., 45200, Akhisar, Manisa, Turkey
[2]Celal Bayar University, Department of Chemistry, 45040,Muradiye, Manisa, Turkey
[3]Ege University,Agriculture Faculty, Department of Field Crops, 35100, Bornova,İ zmir,Turkey

Abstract: Tocopherol homologues of fourteen species of sesame seed oils were determined by reversed-phase HPLC with UV dedection. Sesame (*Sesamum indicum* L.) seeds grown in Turkey contained primarily γ-tocopherol and minor homologues α-and δ-tocopherol, respectively. γ-tocopherol content of sesame seeds varied from 793.0 to 1330.0 mg kg^{-1} ($p<0.01$) whereas seeds contained 1.9-5.1 mg kg^{-1} of α-tocopherol and 0.5-0.6 mg kg^{-1} of δ- tocopherol ($p<0.01$). The total tocopherol content of different samples of sesame seeds varied from 795.8 to 1334.1 mg kg^{-1}. The alterations in tocopherol contents of two varieties of seeds at different roasting temperatures (180,200,220 °C) during 5,10,15,20,25 min in a domestic electric oven was also obtained. The research was rapid for routine analysis of quantitative identification of tocopherols in sesame seed and sesame-based products in food industry.

Key words: Sesame seeds (*Sesamum indicum* L.), tocopherol homologues, rooasting and HPLC

Introduction

Sesame seeds are a valuable oil crop in Turkey. In 1998, Turkey's annual production was 33.500 metric tons and it was the fifth oilseed after cottonseed, sunflower, soy, peanut in agricultural area (Anonymous,1999).

Sesame oil, obtainable from the seeds of (*Sesamum indicum* L.), has been known that is unique owing to its unusually high oxidative stability as compared to other edible oils (Budowski,1962).This strong antioxidant activity has been attributed to the antioxidative sesame lignans, sesamin, sesamolin, sesamol and mainly the presence of γ-tocopherol (Yoshida and Takagi, 1998;Yoshida *et al.*,1995; Yen, 1990 and Yen and Shyu, 1987).

Sesame oil constituents (Yoshida *et al.*,2001 and Özcan and Akgül, 1995 ;Yazioðlu and Karaali, 1983) and the quantification and effectiveness of lipid unsaponifiable matters such as tocopherols and lignans in seed oils has been demonstrated by many investigators (Yoshida and Takagi,1998; Mohamed and Awatif, 1998 and Awatif *et al.*, 1996).

The roasting process is the key step for preparing sesame oil due to the color, chemical composition and quality of sesame oil are all influenced by the roasting conditions (Manley *et al.*, 1972). The optimum roasting parameters on the quality characteristics of sesame oils have been studied (Yoshida and Takagi, 1997 and Yoshida, 1994).

In Turkey it is sold as one of the speciality gourmet oil due to its flavor and stability characteristics and also used in baking foods such as bread, simit ,biscuit, in tahina production as raw material, in special foods with flour,in candy manufacturing and consumed as spice and used in by-products industry. Its oils are used also in soap, cosmetic , pharmaceutical industry and medicinal area.

Five varieties of sesame seeds (*Sesamum indicum* L.) are grown in Turkey. In these sesame population , there are 59% brown, 30% yellow, 13% white, 7% dark-brown and 1% black of varieties(Karabinali,1990). The objective of this study were to determine the tocopherol homologues of fourteen species of sesame seed oils and to quantify the alterations in tocopherol quantities at different roasting conditions by reversed-phase HPLC method.

Materials and Methods

Sesame Seed Material: Fourteen sesame seed samples were collected ($n=14$) as different cultivars at different geographical areas of Turkey. Three sesame (*Sesamum indicum* L.) seed samples used (Gölmarmara, Muganli, Özberk) were obtained from "Aegean Agricultural Research Institute, Menemen" and two samples (Gölmarmara, Özberk) was obtained from Ege University Agricultural Faculty, Department of Field Crops. Three samples grown during the summer period of 2001 (Gölmarmara, Muganli, Çamdibi) were collected from growing area in Antalya. Four samples of sesame seeds was purchased from local market in İzmir and Manisa and production date was the summer of 2001. These above-mentioned samples were brown varieties.Two samples obtained from Marmara area were yellow varieties. The seed species were selected

for uniformity based on seed weight (2.0-2.9 mg). The seeds were sealed in polyethylene bags and stored in -28 °C until needed.
14 varieties of sesame seed samples were analysed as three replicates in all analysis.

Standards: All solvents were the analytical grade (Merck co., Darmstadt, Germany). The tocopherol (Vitamin E) (α, β, γ and δ) standards were purchased from Sigma Chemical Company (St.Louis, MO). All tocopherol standards were of the D-form (*RRR*-).
Calibration curves of standard tocopherol homologues thorough the origin and their R^2 values was 0.9999.

Roasting Conditions of Sesame Seeds: Roasting conditions of sesame seeds were carried out according to Yoshida and Takagi (1997).
Sesame seeds arranged as single layers in Pyrex Petri dishes (12 cm diameter) were roasted using a domestic electric oven at 180, 200, and 220 °C respectively for 5, 10, 15,20, and 25 min. The roasted seed were equilibrated to ambient temperature prior to homogenization for lipid extraction.

Oil Extraction and Sample Preparation: Oil extraction procedure was modified from a described method by Yoshida and Takagi (1997). Uniformited and roasted sesame seeds (150 g for each species) were crushed in a home-made homegenizer with 50 mL of chloroform/ methanol (2:1 v/v) at 0°C and then mixed using vortex for 30s. Homogenized samples was centrifuged at 3500 rpm for 15 min. Chloroform phase containing the extracted lipids was transferred and the residue was extracted with the same procedure three more times and then filtered through lipid-free filter paper. The combined filtrates was concentrated in a rotary evaporator at 30°C under reduced pressure and evaporated using nitrogen flow to dryness. After drying over anhydrous sodium sulphate , final extracts stored in chloroform/methanol (2:1 v/v) solutions in the dark screw caps -28°C until needed. Before injection extract were sonicated for 3 min to remove oxygen and filtered through a 0.50-μm (Acrodisc) filter. 10 μL of filtrate was injected into the HPLC.
Lipids were extracted from unroasted sesame seeds by the same procedures for using as control samples.

HPLC Analysis of Tocopherol Homologues: The analytical isocratic HPLC procedure used was modified from a published method (Yoshida *et al.*,2001). Tocopherol homologues in the seed oils were performed by RP-HPLC using UV detection. HP 1100 ChemStation Software equipped with 5-μm Hypersil-ODS column (250x4.6 mm) [Phenomenex, CAL,USA] using *n*-hexane/ethyl acetate (90:10, v/v) as mobil phase with a flow rate of 1.2 mL/min^{-1}. Tocopherol homologues were monitored with an absorbance dedector set at 296 nm .Dedector sensitivity was 0.05 A.U.F.S and column oven was set at 25°C. The samples were injected as 10 μL. All determinations were performed from three separate extractions and each was injected in triplicate (n = 2).

Analytical Quality Control: The quantitative amount of each compound was calculated by comparison with the peak areas of the standards. Precision of the method of analysis were obtained within the confidence limits (Mean ± 1SD).
Recoveries were determined in duplicate in sesame seed sample (*Gölmarmara*) by spiking pure standards (in range 5-25 μl) to the extraction solutions (100% of the measured content) prior to sample analysis. Mean recoveries were 99.6, 100 and 99.8% for α , γ and δ-tocopherol , respectively.

Statistical Analysis: The data were the mean of three replicates of each samples. Statistical evaluation of the data was conducted by the Statistica for Windows (Statistica, 1998) by one-way analysis of variance "Anova". Duncan's Multiple-Range Test was applied to determine significant differences ($p<0.01$) between mean results.

Results and Discussion

Table 1 shows tocopherol homologues (α, γ and δ-tocopherol) and total tocopherol content of sesame seed oils of fourteen species grown in Turkey (Table 1). γ-tocopherol was the major tocopherol homologue in sesame seed oils($p<0.01$) (Table 1). Tocopherol homologues of sesame extracts were perfectly separated with the our chromatographic conditions. Our performed isocratic HPLC procedure provided rapid baseline separation of α-tocopherol ($t_{retention\ time}$ = 4.7 min), γ-tocopherol ($t_{retention\ time}$ = 7.0 min) and δ-tocopherol ($t_{retention\ time}$ = 9.5 min). α- tocopherol was low concentration followed by δ- tocopherol. γ-tocopherol content of sesame seeds varied from 793.0 to 1330.0 mg kg^{-1} ($p<0.01$) whereas seeds contained 1.9-5.1 mg kg^{-1} of α-tocopherol and 0.5-0.6 mg kg^{-1} of δ- tocopherol ($p<0.01$). There were no significance differences concerning α, δ- tocopherol contents between fourteen samples of sesame seeds ($p<0.01$). No β-tocopherol was detected in sesame seed samples. γ-tocopherol has more potential antioxidant in vegetable oils, although it is a lower vitamin E value in biological systems than other tocopherol homologues (Tokuþoðlu *et al.*, 2001).
The sesame seeds contained 795.8 - 1334.1 mg of

Table 1: Tocopherol homologues of sesame seed (*Sesamum indicum* L.) samples†.

Sesame seed	Dry matter (g 100 g^{-1})	α-Tocopherol (mg kg^{-1})	γ-Tocopherol (mg kg^{-1})	δ-Tocopherol (mg kg^{-1})	Total Tocopherol (mg kg^{-1})
Gölmarmara (Menemen)	95.9	3.6	1330.0	0.5	1334.1
Muganli (Menemen)	95.7	3.2	1072.2	0.5	1075.9
Özberk (Menemen)	95.7	2.9	1023.1	0.6	1026.6
Gölmarmara (Ege Univ.)	95.8	2.2	793.0	0.6	795.8
Özberk (Ege Univ.)	95.5	2.8	1035.4	0.5	1038.7
Gölmarmara (Antalya)	95.6	2.5	1013.0	0.5	1016.0
Muganli (Antalya)	95.6	2.3	812.2	0.6	815.1
Çamdibi (Antalya)	95.4	2.0	895.0	0.6	897.6
Marmara area 1	95.7	1.9	991.3	0.5	993.7
Marmara area 2	95.8	2.3	1008.1	0.5	1010.9
Izmir 1 (Local market)	96.9	5.1	918.2	0.5	923.8
Izmir 2 (Local market)	96.6	4.1	1167.5	0.5	1172.1
Manisa 1(Local market)	95.9	2.9	1253.1	0.5	1256.5
Manisa 2(Local market)	96.9	3.5	973.3	0.5	977.3

†Mean (n = 14) ($p < 0.01$) Each value represents each sample analyzed in triplicate for each variety

Table 2:Tocopherol contents in the oils extracted from sesame seeds roasted at different temperatures in an domestic electric oven†

			Tocopherol			
Sesame seed	Roasting time (min)	Roasting temp. (°C)	α (mg kg^{-1})	γ (mg kg^{-1})	δ (mg kg^{-1})	Total Tocoph. (mg kg^{-1})
Gölmarmara	Unroasted		3.6	1330.0	0.5	1334.1
(Menemen)	5	180	3.4	1319.1	0.4	1322.9
(BROWN)	10		2.9	1303.3	0.3	1306.5
	15		2.1	1284.8	ND	1286.9
	20		1.6	1265.7	ND	1267.3
	25		1.1	1254.5	ND	1255.6
	5	200	3.1	1306.8	0.4	1310.3
	10		2.1	1288.6	ND	1290.7
	15		1.5	1269.7	ND	1271.2
	20		1.1	1250.8	ND	1251.9
	25		0.9	1235.6	ND	1236.5
	5		3.4	1321.4	0.3	1325.1
	10		2.9	1292.5	ND	1295.4
	15		2.0	1280.5	ND	1282.5
Marmara	Unroasted	180	2.3	1008.1	0.5	1010.9
Area(2)	5		2.2	993.7	0.4	996.3
(YELLOW)	10		2.1	986.5	ND	988.6
	15		2.0	971.8	ND	973.8
	20		1.9	954.7	ND	956.6
	25		1.6	945.2	ND	946.8
	5	200	2.3	990.4	0.3	993.0
	10		2.2	973.6	ND	975.8
	15		1.8	962.9	ND	964.7
	20		1.6	949.8	ND	951.4
	25		1.5	932.5	ND	934.0
	5	220	2.2	992.8	0.3	995.3
	10		2.0	978.6	ND	980.6
	15		1.7	957.5	ND	959.2

†Mean (n = 14) ($p < 0.01$) , ND = Not detected
Each value represents each sample analyzed in triplicate for each variety

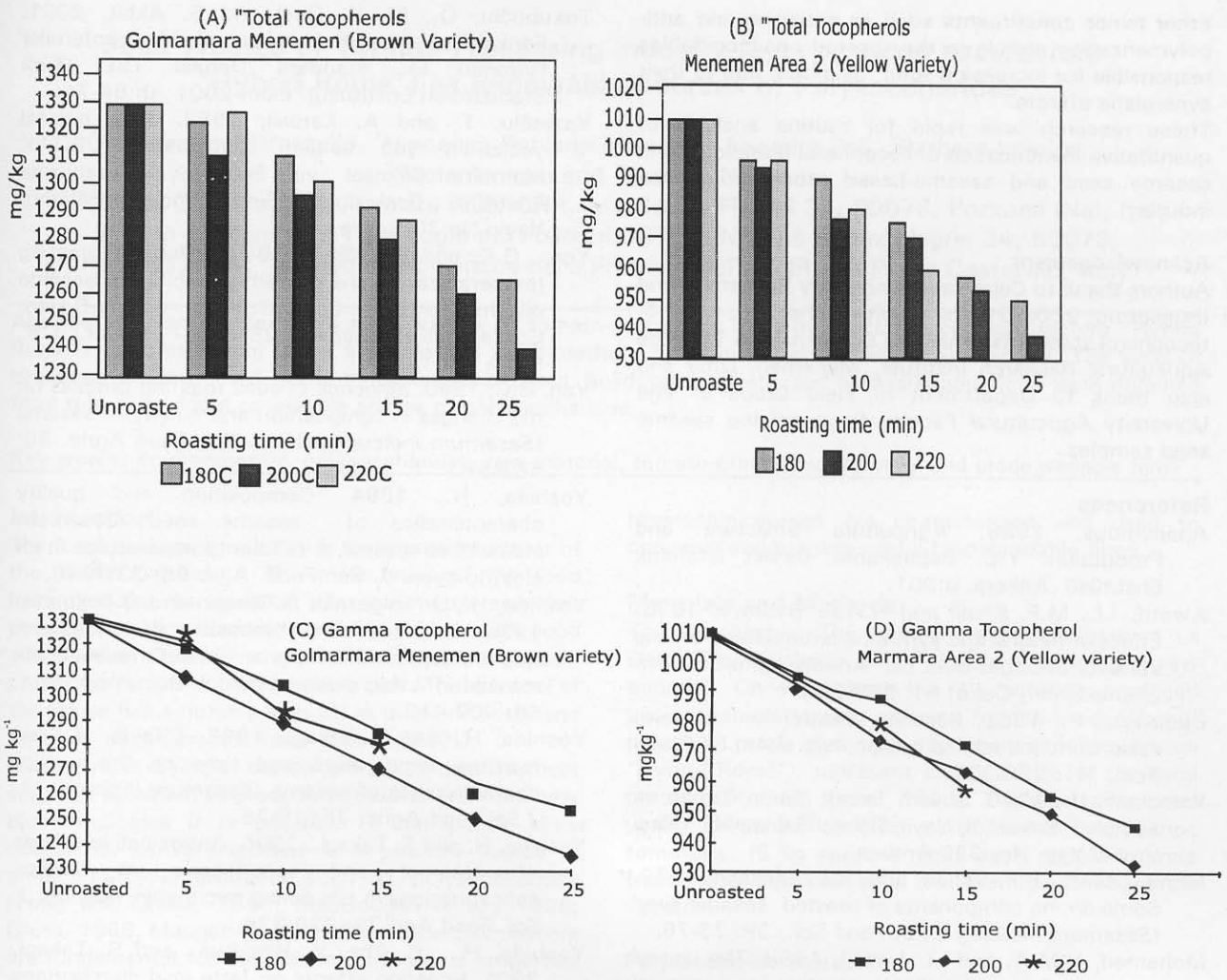

Fig.1: Alterations in total tocopherol levels of roasted brown variety (A), roasted yellow variety (B) and gamma (g) tocopherol levels of roasted brown variety (C), roasted yellow variety (D) of sesame seeds at different temperatures in a domestic electric oven

total tocopherols kg^{-1}. Brown sesame seeds had higher amount of tocopherol (795.8-1334.1 mg kg^{-1}) than yellow seeds (993.8-1010.9 mg kg^{-1}) as shown in Table 1 ($p<0.01$).

Sesame seed samples were roasted at different temperatures (180, 200 and 220°C) using a domestic electric oven. Table 2 shows the alterations in the tocopherol homologues of the oils prepared from sesame seeds roasted at different temperatures ($p<0.01$). γ-tocopherol was still present at more than 85% of its initial value after roasting for 25 min at all temperatures (Table 2).

The levels of total tocopherols remained at over 85% of the original amounts after roasting at 220 C ($p<0.01$) (Table 2 and Fig.1.). Optimal roasting conditions were 5 min at 220°C ($p<0.01$) (Table 2) and the levels of gamma (γ-) tocopherol remained at over 95% of the original amounts in Turkish sesame seeds (Table 2 and Fig.1).

According to our results, one of the unsaponifiable matters such as tocopherols extracted from roasted sesame oil possess antioxidant properties and could be used as alternative natural antioxidants with wide food applications (Table 2 and Fig.1). It can be considered that a combination of a number of sesame lignans mainly sesamin, sesamolin, sesamol , tocopherols and

other minor constituents such as squalene and anti-polymerization sterols in the roasted unsaponifiables responsible for increasing food oxidation due to their synergistic effects.

These research was rapid for routine analysis of quantitative identification of tocopherol homologues in sesame seed and sesame-based products in food industry.

Acknowledgement

Authors thank to Celal Bayar University Research Fund (Project no: 2000/51) for supplying the α, β, γ and δ-tocopherol standards , thank to Dr. Semsettin Tan, Ege Agricultural Research Institute, Menemen, İzmir and also thank to Department of Field Crops of Ege University Agricultural Faculty for supplying sesame seed samples.

References

Anonymous, 1999. Agriculture Structure and Production. *T.C.* Baþbakanlik Devlet İstatistik Enstitüsü ,Ankara. p:301.

Awatif, I.I., M.F. Khalil and H.A.A. Badawy, 1996. Effect of natural and synthetic antioxidants on the stability of virgin olive oil. Annals Agric.Sci., Ain Shams Univ., Cairo. 41: 269-280.

Budowski, P., 1962. Recent research on sesamin, sesamolin, and related compounds. J.Am.Oil Chem Soc., 41: 280-285.

Karabinali, U., 1990. Susam Tarimi, Tarim Orman ve Köyiþleri Bakanliði, Yayin Dairesi Baþkanliði. Çiftçi Üretici Yay. No: 322 Ankara.

Manley, C.H., P.P. Vallon and R.E. Erickson, 1972. Some aroma components of roasted sesame seed (*Sesamum Indicum* L.) J. Food Sci., 39: 73-76.

Mohamed, H.M.A. and I.I. Awatif, 1998. The use of sesame oil unsaponifiable matter as a natural antioxidant. Food Chemistry. 62: 269-276.

Özcan , M. and A. Akgül, 1995. Susam tohumu ve yaðinin bazi bileþim özellikleri. J. of Agri. and Forestry. 19: 59-65.

Statistica, 1998. *User's Guide,* release 6.0; Statsoft; Tulsa, OK.

Tokuþoðlu, Ö., M. K. Ünal and S. Akþit, 2001. Fonksiyonel bir gida bileþeni olarak tokoferoller (Vitamin E). Standard Dergisi TSE (Türk Standardlari Enstitüsü), Ekim-2001 sh:66-74.

Yazioðlu, T. and A. Karaali, 1983. Türk bitkisel yaðlarinin yað asitleri bileþimleri. TÜBİTAK Marmara Bilimsel ve Endüstriyel Araþtirma Enstitüsü , Beslenme ve Gida Teknolojisi Bölümü, Yayin No:70,Gebze.

Yen, G.C. and S.L. Shyu, 1987. Effect of roasting temperature on the oxidative stability of sesame oil. In Trends in Food Science, ed. W.S.Lien. Conference Singapure, 28 Sept.- 20 Oct. (C.F. FSTA 22: 123.

Yen, G.C. 1990. Influence of seed roasting process on the changes in composition and quality of sesame (*Sesamum indicum*) oil. J. Sci. Food Agric. 50: 563-570.

Yoshida, H., 1994. Composition and quality characteristics of sesame seed (*Sesamum indicum*) oil roasted at different temperatures in an electric oven. J. Sci. Food Agri. 65: 331-336.

Yoshida, H., J. Shigezaki, S.Takagi and G.Kajimoto, 1995. Variations in the composition of various acyl lipids, tocopherols and lignans in sesame seed oils roasted in a microwave oven. J. Sci. Food Agri. 68: 407-415.

Yoshida, H. and S. Takagi, 1997. Effects of seed roasting temperature and time on the quality characteristics of sesame (*Sesamum indicum*) oil. J.Sci.Food Agric. 75: 19-26.

Yoshida, H. and S. Takagi, 1998. Antioxidative effects of sesamol and tocopherols at various concentrations in oils during microwave heating. J. Sci. Food Agri.79: 220-226.

Yoshida, H., S. Abe, Y. Hirakiwa and S. Takagi, 2001. Roasting effects on fatty acid distributions of triacylglycerols and phospholipids in sesame (*Sesamum indicum*) seeds. J. Sci. Food Agric. 81: 620-626.