

Original Article

## Gamma Tocopherol Content of Iranian Sesame Seeds

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### Abstract

Gamma tocopherol content of 7 Iranian sesame seeds (*sesamum indicum* L.) was determined by high performance liquid chromatography using C<sub>8</sub> column and methanol, water and butanol as mobile phase. The gamma-tocopherol content varied from 563 to 1095 mg/kg in oil and 293 to 569 mg/kg in sesame seed and was in broad agreement with the Codex range. A large variation was found in the concentration of gamma-tocopherol in oils of different cultivars and the karaj cultivar had more gamma-tocopherol content than the Codex range with significant differences with the other cultivar. The gamma-tocopherol content was almost much more than the other literature values indicating the better nutritional value and oxidative stability in Iranian sesame seed. The present study showed that Iranian sesames are strong radical scavengers and can be considered as good sources of natural antioxidants for medicinal and commercial uses.

**Keywords:** Gamma tocopherol; Sesame seed; HPLC.

### Introduction

Sesame (*Sesamum indicum* L.) seed is one of the most important oil seed crops and have long been categorized as a traditional food in Japan and other East Asian countries (1). Sesame oil is highly resistant to oxidative changes during storage due to high concentrations of various antioxidants in it (2-5). A combination of a number of minor constituents such as tocopherols and sesamol in the sesame seeds could have a synergistic action in increasing the oxidation stability (6). Tocopherols are essential for protection of polyunsaturated fatty acid (PUFA) in plants and animals against oxidative deterioration. They exert their

antioxidant effect by numerous biochemical and biophysical mechanisms, including scavenging active oxygen species and free radicals, and through action as efficient chain terminators in lipid autoxidation reactions (7). Epidemiological evidence for the role of gamma-tocopherol in the development and progression of aging-related diseases has only recently begun to emerge (8-12). Fukuda et al. (13) have suggested that the main active antioxidative constituent in fresh sesame oil extracted from roasted seeds is gamma-tocopherol. In particular, when gamma-tocopherol is given alone to rats, it is only 15% as potent as alpha-tocopherol with respect to vitamin E activity, and corresponding serum levels are 15% of those achieved by a similar dose of alpha-tocopherol (14) but, if gamma-tocopherol, the major tocopherol in sesame

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seeds, is administered by consumption of sesame seeds resulting serum gamma-tocopherol levels are equivalent to alpha-tocopherol (15). Taken together, these data suggest that gamma-tocopherol may be a more effective agent in blocking oxidative damage and that the lower concentration of gamma-tocopherol in plasma may be the result of its higher reactivity. The main objective of this work is to find a good dietary source of gamma-tocopherol for higher protection against oxidative damage and improvement of Iranian nutritional behavior.

## Experimental

### *Reagents and standards*

All solvents were of analytical grade (Merck, Darmstadt, Germany) and were used without further purification. Alpha tocopherol acetate was used as internal standard and purchased from Merck. A stock solution of the gamma-tocopherol (Fluka, Steinheim, Germany) was prepared in n-hexane, kept at -4 °C , protected from light, and diluted to working solutions (100 µg/ml) as necessary. A series concentration (2.5–15 µg/ml) of gamma-tocopherol with alpha tocopherol acetate (15 mg /ml) was prepared for calibration.

### *Sesame seeds*

Seven cultivars of sesame (*Sesamum Indicum* L.) include Branches Naz, Non Branche Naz, Dezful, Darab, Karaj (K-29), Ultan and Varamin were donated from Seed and Plant Improvement Institute during January 2006. The sesame seeds were sealed in a bottle, and stored at 4°C until used.

### *Sesame seed oil*

The oil for raw seeds (5 g) was extracted with Soxhlet apparatus for 4h with n-hexane. The extracted oils were then stored in poly propylene bottles under nitrogen at 20 °C in the dark prior to analysis. A portion of 100 µl oil was diluted with n-hexane in a 10 ml volumetric flask and 100 µl aliquots of a diluted oil was injected to the HPLC column.

### *Analysis*

HPLC analysis was performed with an

analytical HPLC unit included an auto injector (Triathlon, Spark, AJ Emmen, The Netherlands), a HPLC pump (Maxi-Star K-1000 from Knauer, Berlin, Germany), a UV spectrophotometer detector (Knauer, Berlin, Germany), and a computer software (EuroChrom 2000 Version 1.6 from Knauer, Berlin, Germany) as integrator. Analysis of the gamma-tocopherol was carried out by injecting 100 µl aliquots of a diluted oil on the Eurospher100 C<sub>8</sub> Column (4.6 mm× 25 cm). The column was eluted isocratically with the mobile phase (Methanol: Deionized water: Butanol 90: 6: 4) at a flow rate of 1ml /min. The effluent was monitored with the UV detector Set at 294 nm.

## Results and Discussion

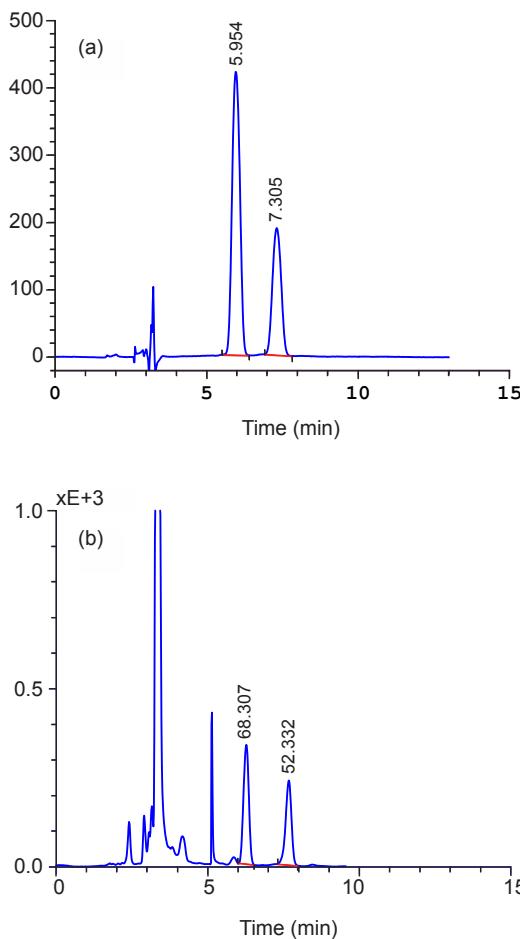
Sesame (*Sesamum indicum* L.) seed, composed of 50% lipid and 20% protein, is one of the important oil seed corps in the world. It is not only a good source of edible oil, but also widely used in baked goods and confectionery products (16). Many scientific studies were conducted to investigate the health-promoting effects of sesame (17-19).

Oxidative stability of sesame oil is superior to that of other vegetable oils (5). Sesamin, sesamolin and sesamol, along with gamma-tocopherol confer superior oxidative to sesame oil as compared to the other sources of vegetable oils (20). Recently the development of novel lipid-containing processed foods has increased; however, rancidity caused by lipid autoxidation poses a serious problem for keeping quality of such systems (21). γ-tocopherol was the only isomer detected in sesame seeds commercial oils and the level of other isomers could be less than  $1 \times 10^{-8}$  g/kg oil (22).

Therefore in the present study the measurement of only γ-tocopherol in sesame oil was undertaken.

### *Characteristics of the HPLC procedure*

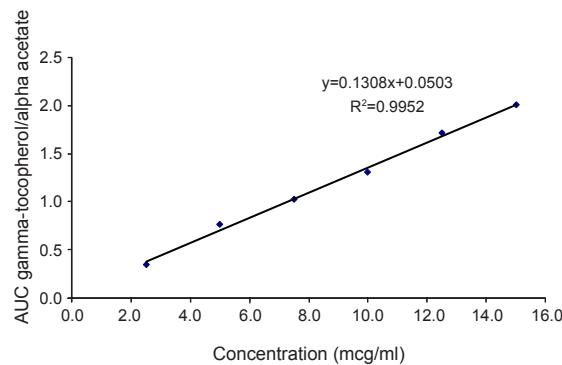
HPLC method for the analysis of tocopherols in plant oil was first described by Van Niekerk (23). HPLC is a one-step technique suitable for quantitative analyses. The HPLC systems used are simple and fast as they involve direct injection of oil solutions.



**Figure 1.** Chromatogram of gama-tocopherol and alpha tocopherol acetate in a) calibration standard solution and b) Non branching Naz cultivar sample.

Figure 1 (a, b) shows a typical elution profile of a diluted sesame seed oil sample and one of the calibration standard solution.

For construction of calibration curve six concentrations of gamma-tocopherol (2.5, 5, 7.5, 10, 12.5, 15 µg/ml) with alpha tocopherol acetate (15 µg/ml) were used. The UV spectrophotometer response was linear for concentration corresponding to 2.5 µg/ml up to 15 µg/ml for gamma-tocopherol and the calibration curve was shown in Figure 2. Assuring the signal to noise ratio should be at least three, the detection limit of the method described was a concentration of approximately 0.5 µg/ml of gamma-tocopherol. The within day precision test was estimated by assaying the gamma-tocopherol content of sesame seed cultivar three times in the same analytical run



**Figure 2.** Calibration curve of standard gamma-tocopherol (2.5-15 µg/ml).

and in order to establish the between-assay precision of the method, sesame seed oils were analysed for gamma-tocopherol on a series of three consecutive days. The results are shown in Table 1.

#### Oil extraction

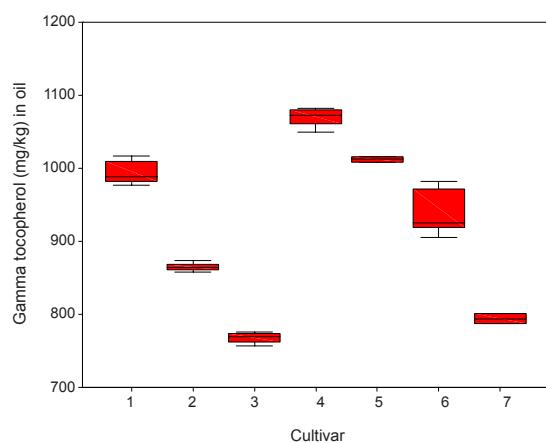
The weights of oil obtained from the soxhlet extraction method were recorded and the oil content of the samples is given in Table 2 as seed oil percentage.

#### Gamma-tocopherol content

The only tocopherol detected in the sesame oil was gamma-tocopherol (23-27), therefore in the present work the gamma-tocopherol content of different sesame seed cultivar was reported (Table 3). The gamma-tocopherol content ranged from 563 to 1095 mg/kg in oil and 293 to 569 mg/kg in sesame seed. The range of gamma-tocopherol in oil was in broad agreement with

**Table 1.** The imprecision test for AUC of gamma-tocopherol in different sesame seed oil cultivars.

Cultivar	CV(%) Between run	CV(%) Within run
Dezfoul	3.192	4.056
Ultan	1.443	0.991
Branching Naz	2.507	1.582
Karaj (K-29)	2.079	1.954
Varamin	2.137	1.920
Darab 14	6.820	2.687
Non Branching Naz	2.332	0.075



1. Dezful, 2. Ultan, 3. Branching Naz, 4. Karaj (k-29), 5. Varamin, 6. Darab 14, 7. Non Branching Naz

**Figure 3.** The gamma-tocopherol content of seven different sesame seed oil cultivars grown in Iran.

the Codex range (521- 983 mg/kg) (24). In 1985 Speek, et al (23) determined the amount of four tocopherol isomers in different oils. The gamma-tocopherol content of sesame oil was  $517 \pm 24$  mg/kg and that of the three other isomers was less than 10 mg/kg. Crews, et al in 2006 (25) reported that the amount of gamma-tocopherol in sesame oil was 430-717 mg/kg and the three other isomers were not detected in the sesame oil. Hemalata, et al (26) in 2004 determined the amount of lignans and tocopherols in Indian sesame cultivars. The gamma-tocopherol (190-500 mg/kg in seed; 400-800 mg/kg in oil) was the only isomer detected in sesame in their work and the level of other isomers could be less than  $10^{-5}$  mg/kg oil. Kamal- Eldin, et al in 1994 (27) also reported the presence of only gamma-tocopherol in sesame oil (584 mg/kg). Wide

**Table 2.** The average of oil content of seven different sesame seed cultivars grown in Iran.

Cultivars	Oil percentage	
	Average%	Interval%
Dezful	51%	50-52%
Ultan	47.2%	46-48.4%
Branching Naz	47.5%	46-49%
Karaj (K-29)	52%	50-54%
Varamin	51.5%	50-53%
Darab 14	49.5%	48-51%
Non Branching Naz	47.5%	46-49%

variation were observed in gamma-tocopherol levels in seeds and oils in this work, but it was almost much more than other literature values (23-27) indicating the better nutritional value and oxidative stability in Iranian sesame seed. We further made a systematic comparison among the gamma-tocopherol content of seven different cultivars with ANOVA test and Dunnett's T3 post Hoc (Figure 3). As it can be seen a large variation was found in the concentration of gamma-tocopherol in oils of different cultivars and Karaj (K-29) cultivar has more gamma-tocopherol content than the Codex range with significant differences with the other cultivar. Also the Branching Naz has the least gamma-tocopherol content among the whole cultivars and the other cultivars had the average values. To our knowledge, the study reported here is the most comprehensive comparison on the gamma-tocopherol content of different sesame seed cultivars. Some sesame seeds had high gamma-tocopherol content and may be rich sources of this valuable antioxidant.

**Table 3.** The gamma-tocopherol content of seven different sesame seed cultivars grown in Iran.

Cultivars	Gamma-tocopherol in sesame oil (ppm) <sup>a</sup>	Gamma-tocopherol in sesame seed (ppm) <sup>a</sup>
Dezful	968.25 ± 32.26	493.81 ± 16.45
Ultan	772.58 ± 11.77	364.66 ± 5.56
Branching Naz	563.65 ± 15.19	417.48 ± 7.22
Karaj (K-29)	1095.31 ± 24.59	569.82 ± 12.28
Varamin	996.07 ± 22.16	512.98 ± 11.41
Darab 14	884.74 ± 63.25	437.95 ± 31.31
Non Branching Naz	618.73 ± 15.43	293.90 ± 7.33

<sup>a</sup> Data are expressed as Mean±SD

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