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Liquid chromatographic determination of α-, β-, γ-, and δ-tocopherol in sesame oils of different origin

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Abstract : The contents of α-, β-, γ-, and δ-tocopherols in sesame oils were determined by high performance liquid chromatography with UV detection. α-Tocopherol contents ranged from 10.28 to 19.79 mg/g oil, β-tocopherol contents from 8.22 to 20.10 mg/g. However, both γ- and δ-tocopherol were less than 1.49 mg/g or not detected. γ-Tocopherol was not detected from both unroasted white and black sesame seed oils. Significantly higher level of tocopherol in sesame oil than other oils is an evidence of the reason why it is highly stable and prevents oxidation. The tocopherol composition for twenty sesame oils was classified by using principal component analysis.

Key words : Tocopherols; Sesame oil; Vegetable oils; Liquid chromatography; Principal component analysis

1. Introduction

Tocopherol (TCP) has been known as one of the natural antioxidants and an important cellular protectant against oxidative damage.[1] The main biochemical function of the TCP is believed to be the protection of polyunsaturated fatty acids against peroxidation.[2] There are four TCP occurring in foods α, β, γ, and δ -derivative, differing in the methylation of the tocol head group. α-Derivative is also known as vitamin E. The ratios of vitamin effectiveness and antioxidant activity of α, β, γ, and δ-TCP are 1.00: 0.50:0.25:0.01, and 1.0:1.3:1.8:2.7, respectively.[3]

TCP occurs naturally in foods such as seeds and nuts, whole grains, vegetable oils, and dark-green leafy vegetables. Vegetable oils have received much attention as an important source of lipids and TCP. Sesame oil has mild and pleasant taste, nutty odour. It is highly stable and resistant to spoiling and oxidation.[4] An advantage of sesame oil is that it is not turned rancid as fast as other oils in hot tropical climates due to its natural antioxidant called sesamol.[5-7] Roasted sesame seed oils are preferably common in eastern Asia because of those characteristic flavours which are formed only during the roasting process. Roasted sesame oil has better resistance to rancidity, due to the antioxidants formed during the roasting of the seed.[8]

All oxidation reactions in lipid containing oils lead to development of off-flavours and off-odours, which render oils unacceptable and shorten their shelf-life. Oxidative reactions decrease nutritional quality, and certain oxidation products are potentially toxic.[9] TCP is a free radical scavenger and singlet oxygen quencher.[10] However, there is a little paper about accurate determination of TCP in sesame oils.[11,12] Therefore, the determination of TCP in sesame oil is very important in ascertaining the durability of oils as well as the oxidative stability of the oil products during storage.

A variety of analytical methods have been used to

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measure TCP as a group or as individual components.[13-17] However, some procedures are time-consuming and require considerable sample preparation. Particularly, gas chromatography (GC) requires saponification, extraction, and derivatization steps, but TCP are very susceptible to oxidation under alkaline conditions.[18] Today, high performance liquid chromatography (HPLC) with UV, fluorimetric or electrochemical detection is the technique used the most frequently for the determination of TCP.[19-23] The major advantage of HPLC is possibility to separate TCP directly without any pretreatment. And HPLC has higher sensitivity and better selectivity than other techniques. Electrochemical detection enables faster and the more sensitive determination of TCP than other method. However, a disadvantage of the electrochemical detection is in the poor resolution of β- and γ-TCP due to their close redox potentials.[24] Strong UV absorbance at 294~298 nm permits UV-based detection of TCP in the sub-nanogram range.

The primary purpose of this paper is to present TCP composition data for 20 different sesame seed oils from several geographic origins and 7 commercial sesame oils in Korea. In this study, UV detection was employed for the determination of α-, β-, γ-, and δ-TCP in vegetable oils. Different sesame oils prepared from varieties of the white and black seeds of which were roasted and unroasted were compared. In addition, differentiation of sesame oils according to their roasting condition was performed by using principal component analysis (PCA) [25-28] on the basis of liquid chromatographic data of TCP compositions. TCP compositions of selected vegetable oils were also investigated to see their differences.

2. Experimental

2.1. Reagents and materials

(±)α-TCP (purity 90%), (+) γ-TCP (purity 90%), and (+) δ-TCP (purity 90%) were purchased from Sigma (St. Louis, MO, USA) as working standards. (±)β-TCP (50 mg/ml in hexane, purity 90%) was from Supelco (Bellefonte, PA, USA). All stock standard solutions (1.25 mg/ml) were prepared by dissolving 25 mg of each working standard in 20 ml hexane. Working standard solutions were prepared by diluting each stock standard solution with hexane. Hexane and isopropanol of HPLC grade were from J.T. Baker (Philipsburg, NJ, USA).

Ten different white or black seeds of *Sesamum indicum* Linne (Pedaliaceae) were collected from different locations in Korea, China, and Japan. And then, sesame oils were obtained by using compression method (60 kgf/cm^2 at 60℃) from 1 kg of pan-roasted (10 min under 170-180℃) or unroasted seeds, respectively. Seven commercial sesame oils and other vegetable oils were purchased from groceries in Seoul, Korea. A sample solution was prepared by dissolving 40 mg of each vegetable oil in 20 ml of hexane. A volume of 10 μℓ of this sample solution was then injected directly onto the liquid chromatograph.

2.2. Liquid chromatography

The HPLC instrumentation was consisted of a LC-980 pump (Jasco, Tokyo, Japan), a Rheodyne injector (model 7161) with a 10 μℓ loop, an UV-975 spectrophotometric detector (Jasco, Tokyo, Japan) equipped with a 10 μℓ volume quartz taper cell (10mm path length), and a C-R6A integrator (Shimadzu, Kyoto, Japan). The separation was performed using a LC-Si column (4.0 mm I.D.×300 mm, particle size 5 ㎛, Supelco, Bellefonte, PA, USA) and hexane:isopropanol(99:1 v/v%) as mobile phase at a flow rate of 1.2 ml/min. A diode-array UV-visible spectrometer (HP 8452A, Hewlet Packard, Palo Alto, CA, USA) was used to obtain spectra of TCP by using working standard solution(2 μg /ml) and hexane as a blank. Finally, detection of TCP was carried out at 295 nm. Peak identification was confirmed in all samples by comparing their retention times with those of known standards. The peak area was measured and from the calibration curve the quantitative amount(mg/g) of TCP in the samples was calculated.

2.3. Principal components analysis

Principal component analysis (PCA) was accomplished with multivariate statistical analysis program (MVSAP, version 4.0) software developed in our laboratory.[25-28]

This software was pre-tested by using the known values and data sets in the literature.[29] From a multivariate data matrix having p variables and n samples, variance-covariance matrix, correlation matrix, eigenvalue, eigenvector, and cumulative proportion for each sample were computed by a personal computer. And then principal component scores were extracted from matrix.

3. Results and discussion

The UV-visible spectra of α-, β-, γ-, and δ-TCP are shown in *Fig*. 1.

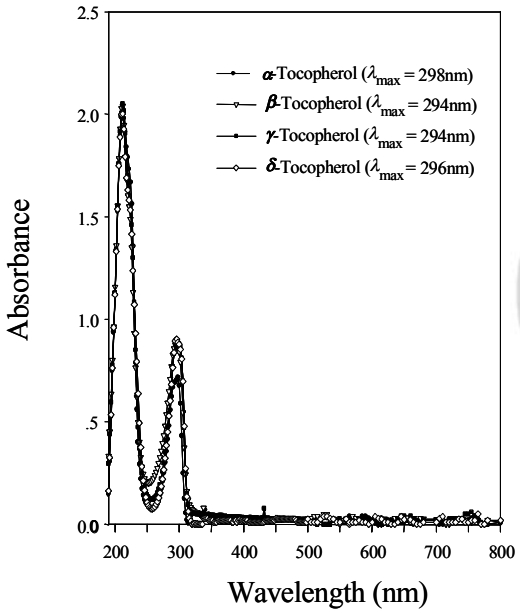

Fig. 1. UV-visible spectra of tocopherol standard solutions (2 μg/ml in hexane)

The maximum absorption wavelengths of those compounds are 298, 294, 294, and 296 nm, respectively. Based on these results, 295 nm was chosen as a detection wavelength for liquid chromatography.

The four peaks of interest were eluted in less than 10 min, as shown in *Fig*. 2. The retention time for α-, β-, γ-, and δ-TCP was 3.93, 5.03, 5.30, and 7.26, respectively. The reproducibility (defined as the relative standard deviation of retention times of 25 replicate runs) of separation for α-, β-, γ-, and δ-TCP was ±1.87, ±3.44, ±6.72, and ±6.33%, respectively. The calibration plots of chromatographic peak area versus the amount of standard TCP ranging from 0.47 ~ 78.13 μg /ml gave the linear regression equations and correlation coefficients (R^2=0.9954~0.9999) as shown in *Fig*. 3.

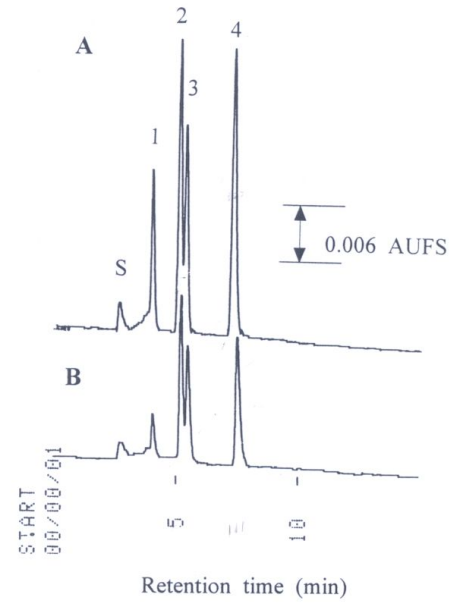

Fig. 2. Typical separation profiles of tocopherol standards by HPLC-UV detection at 295 nm (0.08AUFS), using normal phase Si column(4.0 mm i.d.×300 mm, 5 μm), eluted at 1.2 ml/min with n-hexane / 2-propanol (99:1 v/v%). Peak S, solvent; 1,α-tocopherol; 2, β -tocopherol; 3, γ-tocopherol; 4, δ-tocopherol. sample size 10 μl: A. each 4.88 μg/ml; B. 2.44 μg/ml of standard mixture in n-hexane.

The limit of detection, defined by a signal to noise ratio of 3:1, of α-, β-, γ-, and δ-TCP standard solutions was found to be 0.23, 0.08, 0.47, and 0.09 μg /ml, respectively. The peak area was measured and from the calibration curve the quantitative amount(mg/g) of TCP in the samples was calculated. The reproducibility, defined as relative standard deviation(%) of the method determined by analyzing 10 samples from different batches. The results for α-, β-, γ-, and δ-TCP were

better than ±2.25, ±2.37, ±3.61, and ±4.74%, respectively.

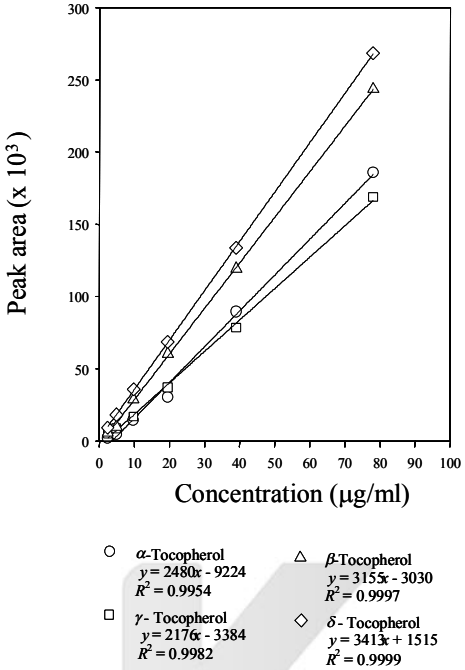

Fig. 3. Calibration curves of tocopherol standard solutions by liquid chromatography with UV detection.

Typical chromatograms for α-, β-, γ-, and δ-TCP in sesame oils are shown in *Fig.* 4. The chromatographic profile was similar for varieties of the white and black seed of which were roasted and unroasted, differing only in the relative concentrations of some TCP. This similarity had been observed in the analysis for varieties of geographic origins. *Table* 1. shows the distribution of α-, β-, γ-, and δ-TCP contents in sesame oils. The feature of compositional patterns is that both α- and β-TCP were abundant in sesame oils, and α-TCP contents ranged from 10.28 to 19.79 mg/g oil, β-TCP contents from 8.22 to 20.10 mg/g. However, both γ-, and δ-TCP were either less than 1.49 mg/g or not detected. The average concentrations of α-, β-, γ-, and δ-TCP in sesame oil were 15.85, 15.16, 0.38, and 0.16 mg/g, respectively. The results obtained in this investigation were different with previous reports by Kamal-Eldin[12] and Speek.[30] Interestingly, γ-TCP was not detected from both unroasted white and black sesame seed oils in

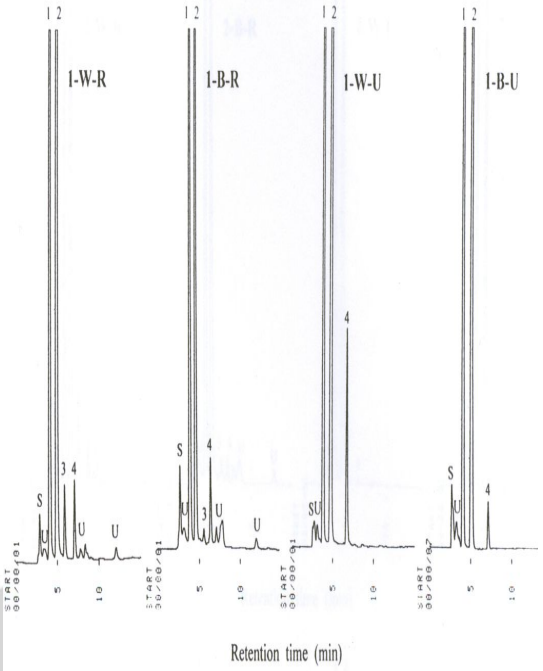

Fig. 4. HPLC chromatograms for tocopherols in sesame oils with UV detection. W-R. white roasted sesame seed; W-U. white unroasted sesame seed; B-R. black roasted sesame seed; B-U. black unroasted sesame seed; Peak S, solvent; 1,α-tocopherol; 2, β-tocopherol; 3, γ-tocopherol; 4, δ-tocopherol; U, unknown.

this study, whereas its level ranged 0.98~1.49 mg/g in white roasted sesame seed oils. These results suggest that the TCP composition is not only related to the species of sesame seeds but to the roasting process. The relative ratios of TCP are summarized in *Table* 2. γ-TCP was identified as a precursor in the synthesis of α-TCP.[31] However, there was no correlation between α-TCP and γ-TCP. The quantitative data of α-, β-, γ-, and δ-TCP contents in seven commercial sesame oils are listed in *Table* 3. γ-TCP was not detected except one sample. The quantitative data of TCP compositions of selected vegetable oils are listed in *Table* 4. Significantly higher level of TCP in sesame oil, compared with other vegetable oils, is possibly an evidence of the reason why it is highly stable and

prevents the breakdown of fatty acids by oxidation.

Table 1 Quantitation of tocopherol composition in sesame seed oils by liquid chromatography with UV detection

Sesame oil	Compound	White seed	
		Roasted	Unroasted
Yechon, Korea	α-Tocopherol	19.2242±0.3536	17.3496±0.6173
	β-Tocopherol	18.9211±0.5090	16.4498±0.5751
	γ-Tocopherol	1.1757±0.0349	n.d.
	δ-Tocopherol	0.3672±0.0348	1.0762±0.0108
Tanyang, Korea	α-Tocopherol	18.2346±0.4150	18.8224±0.8687
	β-Tocopherol	18.0930±0.4466	17.9574±0.8758
	γ-Tocopherol	1.4940±0.1013	n.d.
	δ-Tocopherol	0.2339±0.0221	0.0106±0.0002
Mokpo, Korea	α-Tocopherol	19.7926±0.5798	18.8384±0.7465
	β-Tocopherol	20.1047±0.6237	17.8431±0.7154
	γ-Tocopherol	1.3922±0.0579	n.d.
	δ-Tocopherol	0.2390±0.0192	0.0435±0.0071
Santong, China	α-Tocopherol	15.2460±0.6939	18.4251±0.2250
	β-Tocopherol	15.5261±0.5739	18.4572±0.2226
	γ-Tocopherol	1.1721±0.0909	n.d.
	δ-Tocopherol	0.0955±0.0055	0.2282±0.0082
Fukusima, Japan	α-Tocopherol	15.9750±0.2697	17.9606±0.4054
	β-Tocopherol	18.0270±0.3525	18.4442±0.3452
	γ-Tocopherol	0.9764±0.0946	n.d.
	δ-Tocopherol	0.1026±0.0222	0.1524±0.0187

Mean±s.d.(n=3)
n.d.=not detected

Table 2 Relative ratios of tocopherols in sesame oils

Sesame oil	Ratio	White seed		Black seed	
		Roasted	Unroasted	Roasted	Unroasted
Yechon, Korea	α-T/β-T	1.0160	1.0547	1.2509	1.1353
	γ-T/α-T	0.0612	-	0.0516	-
	γ-T/β-T	0.0621	-	0.0645	-
	δ-T/α-T	0.0191	0.0620	0.0374	0.0124
	δ-T/β-T	0.0194	0.0654	0.0468	0.0141
Tanyang, Korea	α-T/β-T	1.0078	1.0482	1.1685	1.2582
	γ-T/α-T	0.0819	-	0.0491	-
	γ-T/β-T	0.0826	-	0.0574	-
	δ-T/α-T	0.0128	0.0006	0.0035	0.0010
	δ-T/β-T	0.0129	0.0005	0.0041	0.0012
Mokpo, Korea	α-T/β-T	0.9845	1.0558	1.2020	1.1525
	γ-T/α-T	0.0703	-	-	-
	γ-T/β-T	0.0692	-	-	-
	δ-T/α-T	0.0121	0.0023	0.0015	-
	δ-T/β-T	0.0118	0.0024	0.0017	-
Santong, China	α-T/β-T	0.9820	0.9983	1.0961	0.9874
	γ-T/α-T	0.0769	-	-	-
	γ-T/β-T	0.0755	-	-	-
	δ-T/α-T	0.0063	0.0124	0.0022	-
	δ-T/β-T	0.0061	0.0123	0.0024	-
Fukusima, Japan	α-T/β-T	0.8862	0.9738	1.0424	1.0112
	γ-T/α-T	0.0611	-	-	-
	γ-T/β-T	0.0542	-	-	-
	δ-T/α-T	0.0064	0.0085	0.0004	-
	δ-T/β-T	0.0056	0.0082	0.0004	-

Table 3 Quantitation of tocopherols in commercial sesame oils by liquid chromatography with UV detection

Sesame Oil	Amount (mg/g)			
	α-Tocopherol	β-Tocopherol	γ-Tocopherol	δ-Tocopherol
Dongwon	14.1878±0.4073	16.2245±0.4508	n.d.	0.0307±0.0015
Dubaek	3.6306±0.7807	1.4011±0.2408	3.3135±0.2480	n.d.
Haepyo	13.7620±0.1583	16.7537±0.1468	n.d.	0.0146±0.0145
Miwon	14.6705±0.5398	19.0158±0.1468	n.d.	0.0265±0.0043
Ottogi	9.8235±0.1929	16.8551±0.3635	n.d.	0.0314±0.0018
Paeksol	14.4292±0.1939	17.5420±0.1490	n.d.	0.0335±0.0133
Sigol	11.9678±0.0658	17.5371±0.1223	n.d.	0.1534±0.0247

Mean±s.d.(n=3)

n.d.=not detected

Table 4 Quantitation of tocopherols in selected vegetable oils by liquid chromatography with UV detection

Oil	Amount (mg/g)			
	α-Tocopherol	β-Tocopherol	γ-Tocopherol	δ-Tocopherol
Apricot	1.7514±0.0592	1.0133±0.0201	0.9396±0.1170	0.0189±0.0163
Camellia	1.0409±0.0015	0.3992±0.0106	0.4710±0.0039	n.d.
Coconut	0.9275±0.0058	0.2781±0.0072	0.4603±0.0169	n.d.
Olive	1.0206±0.0005	0.2863±0.0255	n.d.	n.d.
Peanut	1.8253±0.2294	1.3198±0.1406	n.d.	0.0086±0.0007
Perilla	1.2105±0.0772	0.8311±0.0285	n.d.	n.d.
Prickly ash berry	1.6336±0.0723	0.6694±0.0408	0.6752±0.0243	0.3707±0.0618
Rape seed	1.3746±0.0147	0.4716±0.0227	n.d.	n.d.
Red pepper	1.6508±0.0210	0.5658±0.0015	0.8868±0.0751	n.d.
Safflower	1.1541±0.1972	0.3427±0.0745	n.d.	n.d.
Soybean	1.0289±0.0171	0.7853±0.2236	n.d.	0.1550±0.0563
Walnut	1.2619±0.1387	0.6455±0.0887	n.d.	0.0183±0.0110
Andong Sesame oil	13.5414±1.3563	15.7917±1.6090	0.4319±0.0162	0.0105±0.0024

Mean±s.d.(n=3)

n.d.=not detected

The data set of TCP composition for twenty sesame oils in *Table* 1. was classified using PCA technique. The 1st, 2nd, 3rd, and 4th eigenvalues were 19.9500, 0.4086, 0.2007, 0.0540, respectively. As shown in *Fig.* 5, the sixty data points of the first two principal components scores (cumulative proportion = 98.76%) by the triplicate analyses for twenty samples are distributed according to different geographical origin, seed color, and roasting condition.

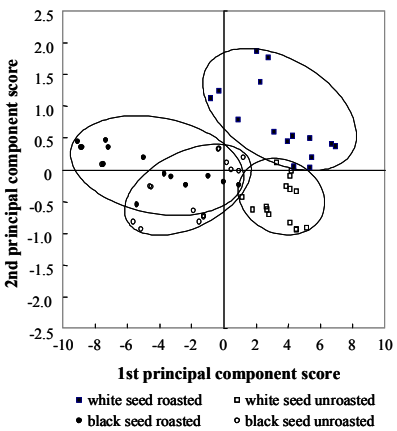

Fig. 5. PCA plot based on tocopherol composition data obtained by the triplicate analyses for twenty samples in *Table* 1.

Acknowledgements

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