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Alpha-Tocopherol Contents of Peanut Seeds Depend on Varieties and Processing Methods

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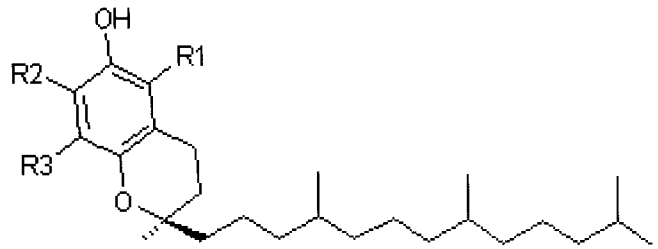
Abstract - The purpose of this study is to establish an extraction and analysis method for α-tocopherol, and to then distinguish among varieties. The α-tocopherol contents of 22 varieties of peanut seeds were analyzed by HPLC. Peanut seeds of cv. Palkwang were processed in two ways, by boiling and roasting. The α-tocopherol contents of the two types of peanuts were compared with fresh seeds without any processing. α-Tocopherol was detected at a retention time of 2.95 minutes. Five standards of α-tocopherol covering a range of 20~100μg/mℓ were made up in 2% isopropyl alcohol/n-hexane and analyzed in duplicate. The α-tocopherol contents differed according to extraction temperature. The contents were 85μg/g or less at 10℃ and 20℃ and 94μg/g at 30℃, but they were decreased at 40℃ or higher. The α-tocopherol contents in 22 peanut varieties were 61.36~96.80μg/g according to variety. Fresh peanuts contained 106.7 μg/g of α-tocopherol, while boiled peanuts contained 108.8μg/g of α-tocopherol, and roasted peanuts contained 109.2μg /g of α-tocopherol.

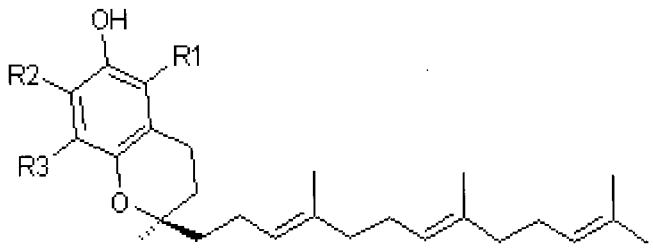
Key words - Peanut, Cultivar, α-Tocopherol, HPLC, Processing method

Introduction

The peanut is the fourth largest edible oil seed crop in the world, lagging just behind the sunflower (*Helianthus annuus* L.). Peanut oil, like any other plant oil, is composed almost entirely of triacylglycerols, a glycerol moiety esterified to three fatty acids. Hence, it contains vitamin E, which is fat soluble. Vitamin E is made up of eight natural compounds, four tocopherols and their corresponding tocotrienols (Fig. 1).

Tocopherol is known to be a natural antioxidant and an important cellular protectant against oxidative damage (Liebler *et al.*, 1996). The main biochemical function of tocopherol is believed to be the protection of polyunsaturated fatty acids against peroxidation (Kamal-Eldin *et al.*, 1996). Of four tocopherols, α-tocopherol is the most active. If α-tocopherol is added during the storage and processing of vegetable oil, it contributes to the stability of oxidation (Lee *et al.*, 1992). In white rats that ingested ethanol over a long period of time, dietary α-tocopherol and α-tocopherol





Tocopherol	R_1	R_2	R_3	Tocotrienol	R_1	R_2	R_3
α-	CH_3	CH_3	CH_3	α-	CH_3	CH_3	CH_3
β-	CH_3	H	CH_3	β-	CH_3	H	CH_3
γ-	H	CH_3	CH_3	γ-	H	CH_3	CH_3
δ-	H	H	CH_3	δ-	H	H	CH_3

Fig. 1. The chemical structures of vitamin E isoforms.

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combined with ascorbate administration may inhibit the formation of liver lipid hydroperoxidation in vivo and were very effective in recovering the liver function in chronically ethanol-treated rats (Lee *et al.*, 1993). The richest natural sources of α-tocopherol are oils from corn, cotton seed, soybean, safflower, and peanut (Ching and Mohamed, 2001).

For the analysis of tocopherol, a colorimetric method, spectrofluorometric method, electrochemical method (i. g. polarography), and chromatography have been used (Strohecker and Henning, 1966; Slover and Lehamann, 1969; Thompson *et al.*, 1972; McBride and Evans, 1973; Waltking *et al.*, 1977; Meijboom and Jongenotter, 1979).

Today, high-performance liquid chromatography (HPLC) with ultraviolet (UV), fluorimetric, or electrochemical detection is the most commonly used technique for the determination of tocopherol (Andreoli *et al.*, 1997; Goffman *et al.*, 1999; Delgado-Zamarreno *et al.*, 2001; Pyka and Sliwiok, 2001; Kim *et al.*, 2002). In the case of high α-tocopherol content, the HPLC method is more effective because it allows for the detection of a higher total content than the colorimetric method (Carpenter, 1979).

Roasted peanuts are available in several different packages. Different coatings can be applied to the peanuts prior to and after roasting to provide a variety of flavors, including honey, smoked, sweet, hot and spicy, and salty taste. Boiled peanuts are popular in Korea where peanuts are common. Fully mature peanuts do not make high quality of boiled peanuts; rather raw or "green" ones are used. "Raw" denotes peanuts in a semi-mature state, having achieved full size, but not being fully dried, as would be needed for roasting. After boiling, they take on a strong salty taste and become softer by cooking time, somewhat resembling a pea or bean, to which they are related.

The purpose of this study is to establish an extraction and analysis method for α-tocopherol, and to then distinguish between varieties. In addition, we investigated the α-tocopherol contents according to processing methods using cv. Palkwnag peanut seeds.

Materials and Methods

Plant materials

Peanut seeds of 22 varieties were obtained from the experimental field of the Honam Agricultural Research Institute. The seeds were cultivated according to standard cultural practices of Rural Development Agriculture, and were harvested on September 10.

Extraction methods

To find the optimal extraction condition, 1g of freeze-dried peanut was homogenized in a blender with 20㎖ of n-hexane and maintained at 5 different temperature from 10 to 50℃ for 5 different times from 10 to 50 minutes, with gentle stirring. The extract was filtered through filter paper (Toyo No. 2) and get rid of solvent by vacuum concentrator. It was filtered through nylon membrane filters (0.45㎛) after solved on 2㎖ mobile phase.

HPLC analysis

α-Tocopherol standard (approximately 99%) was obtained from Sigma Chemical Cooperation. A Dionex LC 20 chromatograph and the absorbance using AD20 with UV-visible detector was used at 295㎚ to detect the α-tocopherol. A Waters μ-Porasil column (3.9× 30㎜, particle size 10㎛) was used at 40 ℃ with a flow rate of 1.0㎖ /min, and 25㎕ of extract was injected to the HPLC chromatograph. Two percent isopropyl alcohol/n-hexane was used as the mobile phase.

Processing

Peanut seeds of cv. Palkwang were processed in two ways, by boiling and by roasting. The former processing method was conducted as follows: 1kg of harvested pods were washed to remove the soil, and were then dried for 2 hours in shaded conditions. These were boiled for 30 minutes in 4 L water (100℃) and subsequently cooled at room temperature. For the latter processing method, 2kg of seeds without pods were roasted for 10 minutes in a cauldron (300℃) and then cooled as described for the boiling method. The α-tocopherol contents of the two types of peanuts were compared with fresh seeds without any processing.

Results and Discussion

In order to detect α-tocopherol in peanuts, the sensitivity of the UV-visible detector system was compared according to the peak area. The five peaks were observed in less than 5 minutes, as shown in Fig. 2, and α-tocopherol was detected at a retention time of 2.95 minutes.

Five standards of α-tocopherol covering a range of 20~100㎍/㎖ were made up in 2% isopropyl alcohol/n-hexane but 2% isopropyl alcohol/n-hexane system showed higher value than n-hexane and was used in our experiment. The constructed calibration curve showed excellent linearity, with a correlation coefficient of

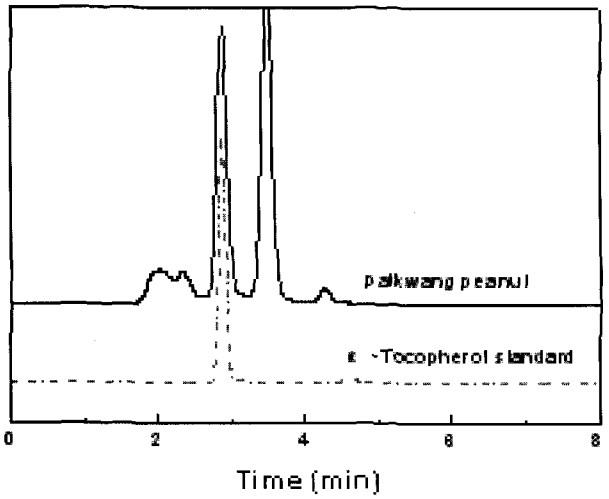

Fig. 2. HPLC chromatogram of α-tocopherol in peanut at 295㎚.

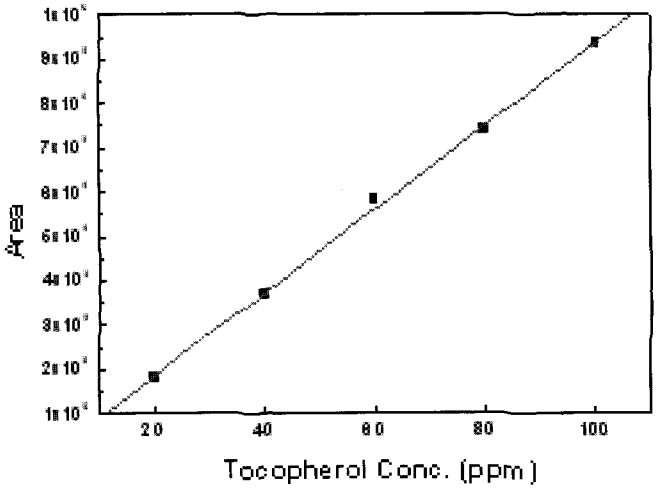

Fig. 3. Calibration curve of α-tocopherol standard solutions by liquid chromatography with a UV-visible detector (r=0.9980).

0.9980 (Fig. 3). The absorbance of each isomer differed according to solvent used. Specifically, α-tocopherol in isopropyl alcohol/n-hexane shows a high contents than that seen for other isomers (Thompson *et al.*, 1972).

The α-tocopherol contents differed according to extraction temperature, being 85㎍/g or less at 10℃ and 20℃and 94㎍/g at 30℃. However, the α-tocopherol contents were found to be decreased at 40℃ or higher (Fig. 4). They also differed according to extraction time. The contents were 80㎍/g and 81 ㎍/g after 10 and 20 minutes, respectively, and were highest, at 87㎍/g, in the case of extraction for 30 minutes. However, this value was decreased in cases of extraction for 40 minutes or longer (Fig. 5).

The α-tocopherol contents in 22 peanut varieties were analyzed after extraction at 30℃ for 30 minutes, ranging from 61.36～96.80 ㎍/g according to variety (Table 1). This result was more than the 10～20㎍/g reported by Park *et al.* (2001), although a similar tendency was shown. We found that the size of peanut seeds caused no significant changes in α-tocopherol contents (Fig. 6).

Fresh peanuts contained 106.7㎍/g of α-tocopherol, while boiled peanuts contained 108.8㎍/g of α-tocopherol, and roasted peanuts contained 109.2㎍/g of α-tocopherol (Fig. 7). Kim and Joo (1994) reported that tocopherol was unstable when it coexisted with unsaturated fatty acids, oxygen, alkali, ultraviolet rays, or metal ions.

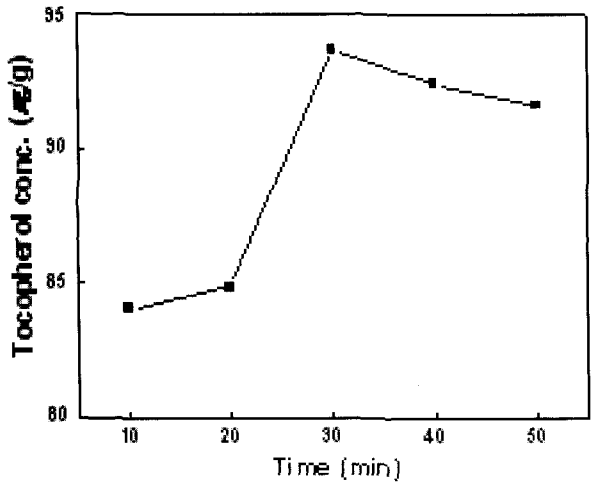

Fig. 4. α-Tocopherol contents in peanut at different extraction times.

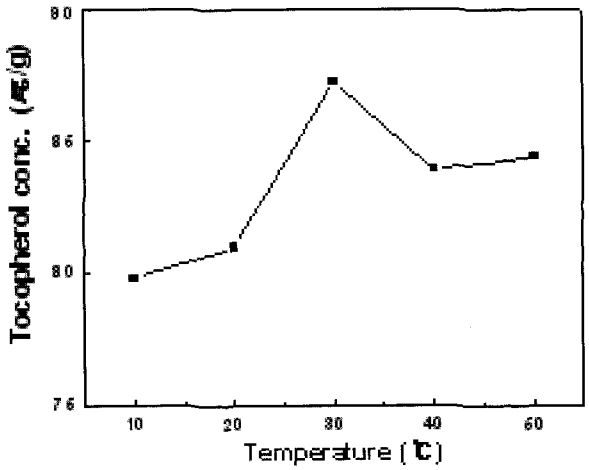

Fig. 5. α-Tocopherol contents in peanuts at different extraction temperatures.

Table 1. α-Tocopherol contents in various peanut varieties

Variety	Botanical type	Seed size†	α-Tocopherol contents (μg/g)
Namkwang	Virginia	Large	76.4
Shinnamkwang			69.3
Hokwang			70.7
Palkwang		Middle	92.6
Daepung			83.3
Dakwang	Spanish	Large	64.8
Shindaekwang			75.4
Daeshin			61.4
Daecheong			79.2
Daeyang			73.3
Bowon			62.7
Joan			76.3
Shinkwang			86.5
Saedle		Middle	77.0
Daekwang			80.0
Mikwang			64.7
Gipung			74.2
Sekwang			64.6
Jokwang		Small	72.8
Wang			96.8
Pungkwang			72.6
Akwang			62.5
LSD (0.05)	-	-	2.1**
CV (%)	-	-	1.8

†Classified based on the investigational standard of Rural Development Administration.
** is significant at 0.01 level.

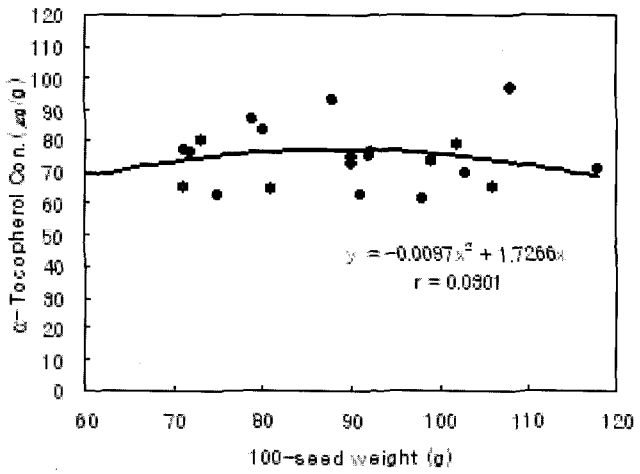

Fig. 6. Correlation between 100-seed weight and α-tocopherol contents in peanut varieties.

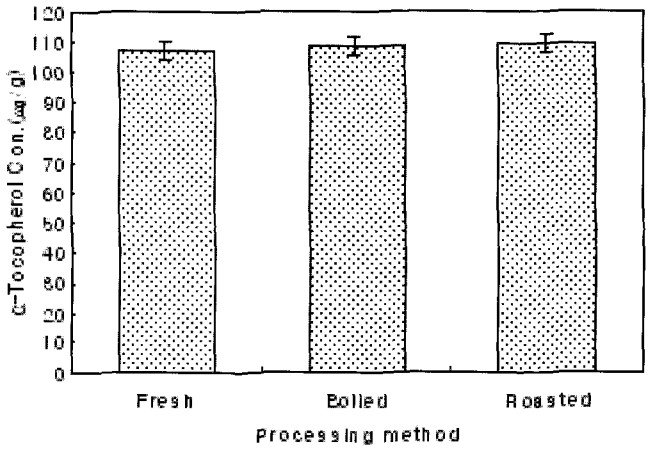

Fig. 7. α-Tocopherol contents according to the peanut processing method.

Literature Cited

Andreoli, R., M. Careri, P. Manini, G. Mori and M. Musci. 1997. HPLC analysis of fat-soluble vitamins on standard and narrow bore columns with UV, electrochemical and particle beam MS detection. Chromatographia 44: 606-612.

Carpenter, A.P. 1979. Determination of tocopherols in vegetable oils. Journal of America Oil Chemistry Socity 63: 328.

Ching, L.S. and S. Mohamed. 2001. Alpha-tocopherol content in 62 edible tropical plants. J. Agric. Food Chem. 49: 3101-3105.

Delgado-Zamarreño, M.M., M. Bustamante-Rangel, A. Sánchez-Pérez and J. Hernández-Méndez. 2001. Analysis of vitamin E isomers in seeds and nuts with and without coupled hydrolysis by liquid chromatography and coulometric detection. J. Chromatography A. 935: 77-86.

Goffman, F.D., L. Velasco and W. Thies. 1999. Quantitative determination of tocopherols in single seeds of rapeseed (*Brassica napus* L.). Fett/Lipid 101: 142-145.

Kamal-Eldin, A., L. Lars-Åke and L. Appelqvist. 1996. The chemistry and antioxidant properties of tocopherols and tocotrienols. Lipds 31(7): 671-701.

Kim, S.O., N.S. Kim, B. S. Noh and D.S. Lee. 2002. Liquid chromatographic determination of α-, β-, Υ-, and δ-tocopherol in sesame oils of different origin. Analytical Science & Technology 15(3): 248-255.

Kim, U.K. and K.J. Joo. 1994. EPA, DHA and tocopherols contents in fish oil products and fishes. J. Korean Soc. Food Nutr. 23(1): 68-72.

Lee, C.H., Y.J. Jung, D.K. Park, C.W. Kim, Y.B. Han, W.C. Lee and J.B. Kim. 1993. Effects of ascorbate and α-Tocopherol administration on liver function in chronically ethanol-treated rats. J. Korean Soc. Food Nutr. 22(2): 132-137.

Lee, I.B., K.J. Choi, K.K. Yu and K.W. Chang. 1992. Tocopherols and fatty acids in plant seeds from Korea. J. Korean Agric. Chem. Soc. 35(1): 1-5.

Liebler, D.C., J.A. Burr, L. Philips and A.J.L. Ham. 1996. Gas chromatography-mass spectrometry analysis of vitamin E and its oxidation products. Anal. Biochem. 236: 27-34.

McBride, H.D. and D.H. Evans. 1973. A rapid voltammetiric method for the determination of tocopherol and antioxidants in oil and fats. Anal. Chem. 45(3): 446-449.

Meijboom, P.W. and G.A. Jongenotter. 1979. A quantitative determination of tocotrienols and tocopherols in palm oil by TLC-GLC. J. Am. Oil Chem. Soc. 56(1): 33-35.

Park, M.R., S.H. Baek, Y.K. Cheong, M.H. Lee, K.G. Choi and S.J. Yun. 2001. Tocopherol content and composition in peanut. Korean J. Crop Sci. 46(4): 279-283.

Pyka, A. and J. Sliwiok. 2001. Chromatographic separation of tocopherols. J. Chromatography A. 935: 71-76.

Slover, H.T. and J. Lehamann. 1969. Vitamin E in foods: determination of tocopherols and tocotrienols. J. Am. Oil Chem. Soc. 46(8): 417-420.

Strohecker, R. and H.M. Henning. 1966. Vitamin assay-tasted methods. CRC press. Cleveland. pp. 283-308.

Thompson, J.N., M. Erodody and W.B. Maxwell. 1972. Chromatographic separation and spectrophotofluorometric determination of tocopherols using hydroxyalkoxypropyl sephadex. Anal. Chem. 50: 267-280.

Waltking, A.E., M. Kiernan and G.W. Bleffert. 1977. Evaluation of rapid polarographic method for determining tocopherols in vegetable oils and oil-based products. JAOAC 60(4): 890-894.

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