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Tocopherol Content and Composition in Peanut

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ABSTRACT: The content and composition of tocopherol were analyzed in peanuts produced in Korea. The tocopherol assay was consisted of an extraction with n-hexane:isopropanol mixture followed by high pressure liquid chromatography with fluorescence detection. Tocopherol content was higher in leaves and seeds but lower in seed coats and shells. α-/γ-Tocopherol ratio was as low as 0.53 in seeds and as high as 22.5 in shells. Tocopherol content in seeds of nine varieties ranged from 142 to 220 ng/mg dry weight, and the α-/γ-tocopherol ratio from 0.40 to 0.75. Tocopherol content decreased by 18% but α-/γ-tocopherol ratio increased by 44% in roasted seeds. The results indicate that the level of tocopherol in Korean peanut varieties is moderate and the ratio of α- to γ-tocopherol is low.

Keywords : peanuts, tocopherols, α-/γ-tocopherol ratio

In 2000, about 12,400 Mt of peanuts were produced from 6,800 ha of cultivation area in Korea (FAO, 2001). Peanuts are mostly consumed as favorite foods and roasted peanuts are the most common product for human consumption in Korea. As the concept of functional food is widely accepted, phytochemicals beneficial for health call for attention in research and industrial communities. It has been known that peanut seeds contain health-promoting phytochemicals such as tocopherols.

Tocopherols are lipid soluble antioxidants known collectively as vitamin E. Four isoforms of tocopherols, α-, β-, γ-, and δ-tocopherol, are synthesized in plants and other photosynthetic organisms. The isoforms differ by the numbers and positions of methyl substituents on the aromatic ring of the molecules (Hess, 1993). The major tocopherols found in human diets are γ- and α-tocopherol (Sheppard & Pennington, 1993). α-Tocopherol from the natural sources (*R,R,R*-α-tocopherol) is the most biopotent form of vitamin E (Machlin, 1991).

Clinical and epidemiological evidences suggest that vitamin E decreases the risk of cardiovascular disease and cancer, promotes immune system, and prevents or slows various chronic degenerative diseases and aging (Pryor, 2000; WCRF, 1997). As the evidiences on health benefits from vitamin E accumulate, synthetic forms of tocopherols such as tocopheryl acetate have become commercially available as vitamin E supplements. Synthetic tocopherols are also widely used in food industry as antioxidants or preservatives (Sheppard & Pennington, 1993).

Seeds of oil-seed crops such as peanuts are important sources of vitamin E in human diets. Content and composition of tocopherols in peanut seeds and oils differ considerably depending on genotypes and production sites (Fore *et al.*, 1953; Sanders *et al.*, 1992). In general, peanut seeds contain about 114 and 84 ng/mg of α- and γ-tocopherol, respectively. The nutritional quality of tocopherols in peanut seeds and oil is poor due to the low α- to γ-tocopherol ratio which ranges from 0.6 to 1.36 (Sheppard & Pennington, 1993).

One of the ways to promote peanut consumption is to improve its nutritional and functional quality to meet consumers health interests. Due to the beneficial effects of vitamin E, α-tocopherol is becoming one of the major characters for improvement through conventional breeding and even through metabolic engineering (Sintani & DellaPenna, 1998). However, there is no information on tocopherols in peanuts produced in Korea. Information on composition and content of tocopherols is critical for efficient breeding and utilization of peanuts. Therefore, this study was conducted to develop baseline data on tocopherol content and composition in peanuts grown in Korea.

MATERIALS AND METHODS

Nine peanut varieties were used in this experiment: Namkwangtankong, Namdaetankong, Daekwangtankong, Saedletankong, Iksantankong, Jinpoongtankong, Palkwangtankong, Degona, and Yudereka. Peanuts were grown in the experimental field at Chonbuk National University, Chonju, Korea in 2000, according to the standard cultivation guideline from

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RDA, Korea. Young leaves were sampled at early growth stage and kept at -70°C. Mature seeds were harvested, air-dried and stored at 4°C. To examine heat stability of tocopherols, some seeds were roasted on a pan for 5 min at 140°C with gentle agitation and kept at -70°C with seed coats removed. For tocopherol analysis, tissues were ground into fine powder under liquid nitrogen. The ground tissue was freeze-dried and used immediately for tocopherol extraction. Seed coats were separated from embryos and endosperms, and were analyzed separately.

Tocopherols were analyzed according to Tanaka *et al.* (1999) with some modifications. Samples were protected from light during the analysis process. The freeze-dried tissue powder was added in n-hexane:isopropanol (98 : 2) and mixed by vortexing vigorously for 1 min. After incubation for 5 min at room temperature, the mixture was centrifuged at 12,000*g* at 4°C for 5 min. The supernatant was filtered through 0.45 μm syringe filter and the filtrate was used for HPLC analysis on a μPorasil column (3.9× 300 mm, Waters, USA). Tocopherols were eluted with n-hexane:isopropanol (98 : 2, v/v) at a flow rate of 1.0 ml/min. Tocopherols were detected using a fluorescence detector (Waters 474, Milford, USA) set at 295 nm as an excitation wavelength and at 325 nm as an emission wavelength. Analysis for the samples was replicated three times. Tocopherol standards were purchased from Merck (Darmstadt, Germany). ANOVA and LSD tests were conducted for the data collected from 3 replications using the SAS program.

RESULTS AND DISCUSSION

The three isoforms of tocopherols, α-, γ-, and δ-tocopherol, were extracted and detected by the procedure employed in this experiment (Fig. 1). The coelution of the peaks from the extracts of peanut seeds with the standard tocopherols strongly indicated that the compounds in the extract were tocopherols. Retention times for α-, γ-, and δ-tocopherol were 4.3, 5.6, and 7.0 min, respectively. In peanut seeds, γ-tocopherol was most abundant followed by α-tocopherol. Only trace amount of δ-tocopherol was detected in peanut seeds. This result is constant with the previous observations on tocopherols in peanuts (Fore *et al.*, 1953; Sanders *et al.*, 1992).

The extraction procedure is a critical step in tocopherol analysis. The solvents used for tocopherol extraction include ether, hexane, acetone, chloroform, ethanol, propanol, and in some cases, combinations of solvents. The AOAC method for pharmaceuticals use n-hexane as the extracting solvent (Cunniff, 1995). Therefore, n-Hexane:isopropanol (98:2) was used as an extracton solvent in this experiment. Saponification is often used in the extraction procedure to remove

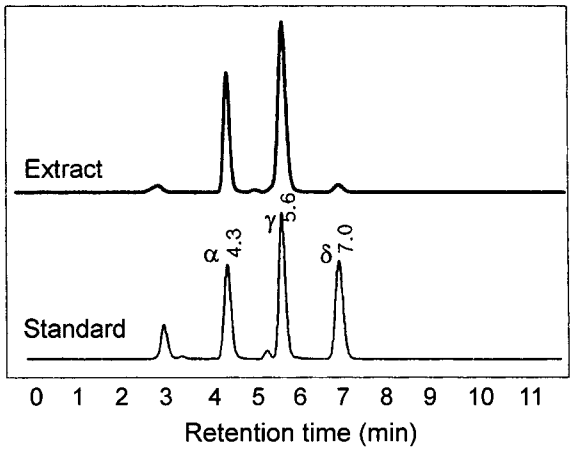

Fig. 1. Chromatograms of an extract of peanut seeds and the tocopherol standards. Retention time for α-, γ-, and δ-tocopherol was 4.3, 5.6, and 7.0 min, respectively. Tocopherol extract of peanut seeds and tocopherol standard was seperated on a normal phase column with n-hexane : isopropanol (98 : 2, v/v) at a flow rate of 1.0 ml/min. Tocopherols were detected using a fluorescence detector set at 295 nm as an excitation wavelength and at 325 nm as an emission wavelength.

interfering substances prior to tocopherol determination (Eitenmiller & Landen, 1999). However, extensive saponifications can cause oxidative degradation of tocopherols (Piironen *et al.*, 1991; Speek *et al.*, 1985). Furthermore, a simple extraction method was successfully used for tocopherol analysis in plants and aquatic organism samples (Lee, 1992; Huo et al., 1996; Tanaka et al., 1999). A specific and less-time consuming procedure for tocopherol analysis is required especially for the screening of a large number of genetic materials. The analysis procedure employed in this study could be applicable in germplasm evaluation for tocopherols.

Tocopherol content and composition in peanut tissues were determined in a variety, Jinpoongtankong. Total tocopherol content (α- + γ-tocopherol) was about 300.6, 220.2, 8.5, and 4.2 ng/mgDW in leaves, seeds, seed coats, and shells, respectively (Fig. 2A and B). Total tocopherol content in leaves was about 37% higher than in seeds without seed coats. Total tocopherol content in leaves and seeds was 35- to 72-fold higher than that in seed coats, and 26- to 52-fold higher than that in shells (Fig. 2A and B).

Composition of tocopherols was also different among tissues as indicated in the α-/γ-tocopherol ratios. The ratio was 4.2, 0.5, 2.9 and 22.5 in leaves, seeds, seed coats, and shells, respectively (Fig. 2C).

No information on tocopherols in peanut tissues other than seeds is available. The occurrence of tocopherol in non-chlorophyll-containing tissues such as roots and seeds as

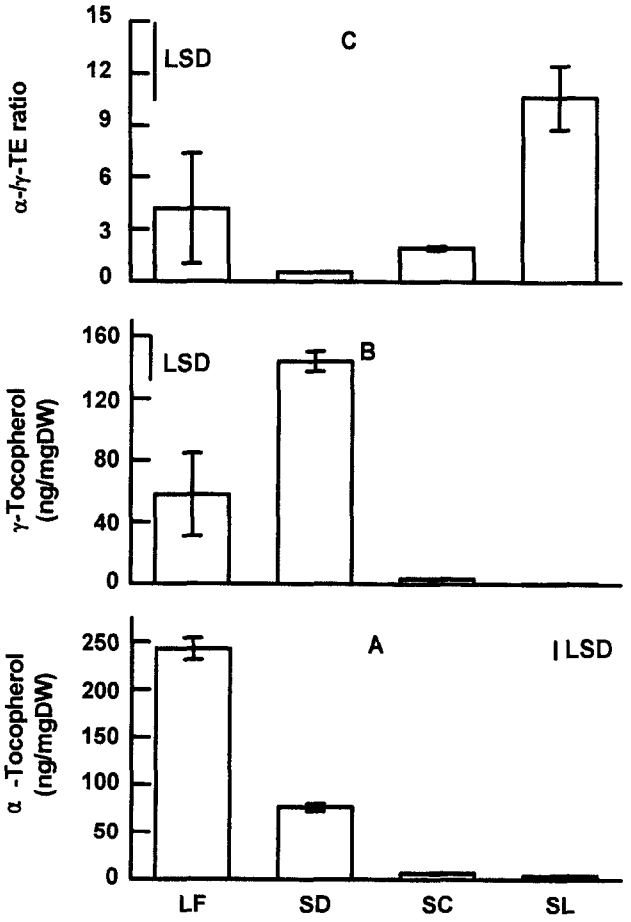

Fig. 2. Tocopherol content and composition in peanut tissues. α-Tocopherol (A) and γ-tocopherol (B) content, and the ratio of γ-tocopherol to γ-tocopherol (α-/γ-TE ratio) (C) in leaf (LF), seed (SD), seed coat (SC), and shell (SL) of variety Jinkwang. LSD values for α- and γ-tocopherol content, and the α-/γ-TE ratio were 14.0, 31.79, and 5.04, respectively.

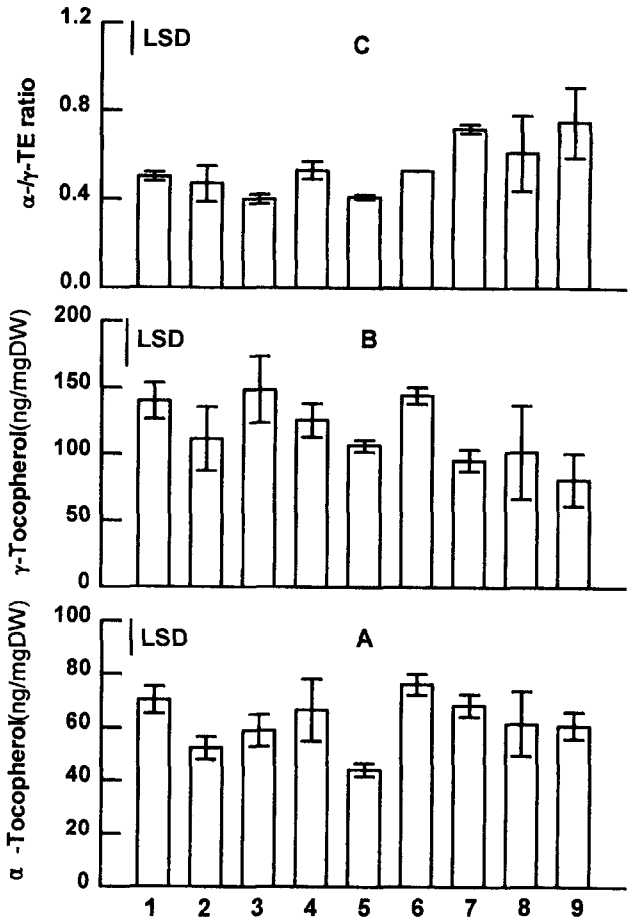

Fig. 3. Tocopherol content and composition in seeds of the 9 peanut varieties. α-Tocopherol (A) and γ-tocopherol (B) content, and the ratio of α-tocopherol to γ-tocopherol (α-/γ-TE ratio) (C). Varieties used were: 1(Namkwangtankong), 2(Namdaetankong), 3(Daekwangtankong), 4(Saedletankong), 5(Iksantankong), 6(Jinpoongtankong), 7(Palkwangtankong), 8(Degona), and 9(Yudereka). LSD values for α- and γ-tocopherol content, and the α-/γ-TE ratio were 14.56, 39.97, and 0.18, respectively.

well as green leaves has been reported with variable α-/γ-tocopherol ratios in other plant species. In general, green tissues contain higher levels of tocopherol and α-/γ-tocopherol ratio than non-chlorophyll-containing tissues such as seeds and roots in most plants (Hess, 1993). Our result also indicates that leaves as well as seeds are rich sources of tocophrols in peanuts. It is interesting that the α-/γ-tocopherol ratio in shells is higher than in leaves even though total tocopherol content in shells is very low.

Tocopherol content and composition of peanut seeds varied moderately among the varieties analyzed. Total tocopherol content ranged from 142 to 220 ng/mgDW with the average of 179 ng/mgDW. Tocopherol content was higher in Jinpoongtankong and Namkwangtankong but lower in Iksantankong and Yudereka (Fig. 3A and B). γ-Tocopherol content was 25 to 60% higher than α-tocopherol as reflected in the low α-/γ-tocopherol ratios which ranged from 0.40 to 0.75 with an average of 0.55. The ratio was higher in Palkwangtankong and Yudereka, but lower in Daekwangtankong and Iksantankong (Fig. 3C). The variety Iksantankong was distinguished by the low tocopherol content and α-/γ-tocopherol ratio. The varieties with a higher tocopherol content such as Jinpoongtankong and Namkwangtankong had the α-/γ-tocopherol ratio of 0.53 which is lower than the average. No clear relationship was found between the total content and α-/γ-tocopherol ratio.

Peanuts are rich sources of tocopherols but information on tocopherol content in peanuts produced in Korea is rarely available. Content and composition of tocopherols in peanut seeds and oils differ considerably depending on genotypes and production sites (Fore *et al.*, 1953; Sanders *et al.*, 1992).

In general, peanut seeds contain about 198 ng/mgDW of tocopherols with the α-/γ-tocopherol ratio of around 0.8. Peanut oils contain tocopherols at about 244 ng/mg but with the lower α-/γ-tocopherol ratio of 0.6 (Sheppard & Pennington, 1993). Tocoperol content also varies between 268 and 510 ppm in peanut oils (Young, 1996). The nutritional quality of tocopherols in peanut seeds and oil is poor due to the low α-/γ-tocopherol ratio which ranges from 0.6 to 1.36 (Sheppard & Pennington, 1993). Therefore, our results indicate that tocopherol content in peanuts of domestic production is at the similar levels as reported in peanuts produced in other countries. However, the α-/γ-tocopherol ratio is lower than the levels reported elsewhere (Sheppard & Pennington, 1993; Young, 1996).

Varietal variances in tocopherol content and composition have also been indicated in several oil seed crops including peanuts (Fore *et al.*, 1953; Sanders *et al.*, 1992; Young, 1996). The low α-/γ-tocopherol ratios of seeds and oils indicate low vitamin E activities relative to the total tocopherol content. Compared with soybean, the content of tocopherol is about 4-fold lower but the ratio of α-/γ-tocopherol is about 6-fold higher in peanut oil (Grusak, 1999; Sheppard & Pennington, 1993). Varietal differences detected in the tocopherol content and α-/γ-tocopherol ratio in peanut seeds indicate that these traits can be improved through breeding efforts.

Tocopherols were analyzed in the roasted seeds with seed coats removed to estimate the effect of roasting on tocopherol content and composition. Two varieties were selected based on the α-/γ-tocopherol ratio, Daekwangtankong for the lower ratio and Palkwangtankong for the higher ratio. After roasting, total tocopherol contents decreased by 38% and 8% in Daekwangtankong and Palkwangtankong, respectively (Fig. 4A and B). The average tocopherol content decreased from 185 to 149 ng/mgDW by roasting. The α-/γ-tocopherol ratio, however, increased in both varieties by 57 and 31% in Daekwangtankong and Palkwangtankong, respectively (Fig. 4C).

Lower tocopherol content and composition in oil than in seeds indicate that tocopherol content and composition is affected by refining processes (Young, 1996). Therefore, it is assumed that content and composition of tocopherol may also be affected by roasting. Our results indicate tocopherol content decreases but the α-/γ-tocopherol ratio increases by roasting. It is reported that roasted peanuts contain about 106 ng/mgDW of α-tocopherol which is the similar level as in our results (FCT, 1991). Thus, our results suggest that in general γ-tocopherol is more unstable at high temperature than α-tocopherol in peanut. More detailed study is needed to address the differential sensitivity of tocopherol isoforms to heat because the heat liability is related to the processing property in peanuts.

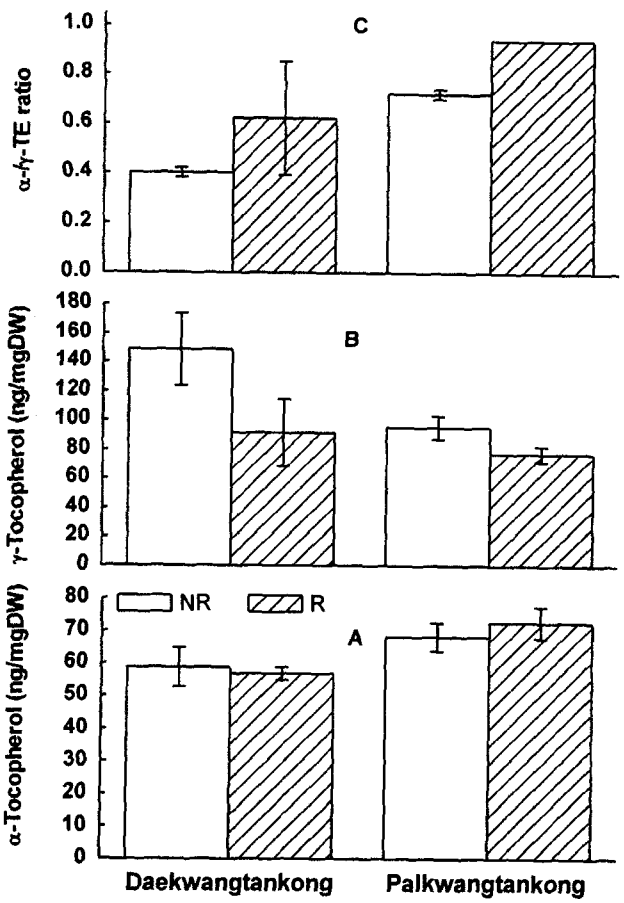

Fig. 4. Effect of roasting on tocopherol content and composition. α-Tocopherol (A) and γ-tocopherol (B) content, and the ratio of α-tocopherol to γ-tocopherol (α-/γ-TE ratio) (C) in non-roasted (NR) and roasted (R) seeds of Daekwangtankong and Palkwangtankong. There was significant difference between variety in tocopherol contents and the ratio. Significant effect of roasting was indicated in the γ-tocopherol content (Pr>0.02) and α-/γ-TE ratio (Pr>0.01).

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