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Trans-Resveratrol Contents of Peanut Seeds Depend on Varieties and Processing Methods

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Abstract - The high-performance liquid chromatography (HPLC) method for the determination of *trans*-resveratrol in 34 germplasms and processing methods of peanut seeds has been modified. Peanut germplasms contained *trans*-resveratrol contents of 0.14~4.96μg/g, but findings for the testa color were not significant. However, two germplasms, 'KIGAN' and 'CS1', contained more *trans*-resveratrol contents than the other germplasms. The contents of their were 2.26μg/g and 4.96μg/g. The tested processing methods caused no significant changes in *trans*-resveratrol contents. The contents of fresh, boiled, and roasted peanuts were 0.36, 0.32, and 0.40μg/g, respectively in cv. Palkwang, and 0.22, 0.22, and 0.26μg/g, respectively, in cv. Jakwang. Differences were not significant among fresh, boiled, and roasted peanuts. The grains of 'Palkwang' and 'Jakwang' contained *trans*-resveratrol contents of 0.34μg/g and 0.24μg/g, and testa contained 1.12μg/g and 1.00μg/g, respectively. However, when comparing absolute quantity, the *trans*-resveratrol contents appears to be approximately 3~4 times higher in the testa than in the grain of the peanut, although the total contents were not different because the ratio of testa was low in peanut seeds.

Key words - Peanut, *trans*-Resveratrol, HPLC, Germplasm, Testa color, Processing method

Introduction

Peanuts (*Arachis hypogaea* L.) have a heat value of high calories, and contains high lipid (40% or more) and protein (20% or more) contents. Thus, it has been widely used as a source of edible oil, butter, margarine, confectionery, manufacturing, and machine oil (Cho, 1993). The methanol extract of the seed coat of the peanut has an anti-mutagenic effect (Duh *et al.*, 1992), and peanuts contain resveratrol, which has been shown to possess cancer chemopreventive activity in mice and to act as not only an antioxidant and anti-mutagen (Jang *et al.*, 1997), but also as an antimicrobial (Chan, 2002). In addition, peanuts contain various functional materials, including 4-hydroxycinnamic acid, 3-methoxy-4-hydroxycinnamic acid, 3, 4-dihydroxybenzoic acid (Wee and Park, 2000), peanut lectin (Lotan *et al.*, 1975), luteolin (Duh and Yen, 1995), vanillin (Sobolev, 2001), and tocopherol (Park *et al.*, 2001).

Resveratrol (*trans*-3, 5, 4'-trihydroxystilbene) is known to exist in wine in the aglycon and glycoside forms, which is referred to as resveratrol-3-O-β-D-glucoside when found in grape products and usually is present at significantly higher concentrations than the aglycon. It exists in the *cis* and *trans* isomeric forms, and a 3-β-glycoside, piceid (Mattivi *et al.*, 1995), all of which are physiologically important. This material is converted from p-coumaroyl-Co A to the major resveratrol products by stilbene synthase (STS). The phenolic compound, resveratrol, is an non-flavonoid phytoalexin that is produced by plants in response to fungal infection or stress (Langcake, 1981). STSs were rarely found in higher plants, and occur in distantly related species such as *Arachis hypogaea* (Schoppner and Kindl, 1984), *Vitis vinifera* (Sparvoli *et al.*, 1994), *Pinus sylvestris* (Schanz *et al.*, 1992), *P. strobus* (Raiber *et al.*, 1995), and *Rhei rhizoma* (Kashiwada *et al.*, 1998).

Resveratrol has been found in peanut hypocotyl (Ingham, 1976) and germination seeds (Keen, 1975; Arora and Strange, 1991), but only after microorganism inoculation and wounding (Chung *et al.*, 2001). Its level depends on a number of factors such as variety, geographical location, climate condition, fungal infection, ultraviolet light exposure, and enological techniques in grape (Siemann and Creasy, 1992; Jeandet *et al.*, 1995; Romero-Pérez *et al.*, 1996). In general, the induction of resveratrol synthesis has been demonstrated by pathogen infection, fungal cell wall elicitors, and UV light in peanut and grapevine suspension cells (Keen and Ingham, 1976; Fritzemeier *et al.*, 1983). Lanz *et al.* (1990) and Chung *et al.* (2001) reported that resveratrol is accumulated through biotic and abiotic stresses such as fungal infection, elicitor, and UV light.

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This study was conducted to search the germplasm containing high *trans*-resveratrol contents. In addition, we investigated the *trans*-resveratrol contents according to processing methods using cultivar peanut seeds, 'Palkwang' and 'Jakwang'.

Materials and Methods

Plant materials

The concentration of *trans*-resveratrol was determined in 34 germplasms, 14 black testa peanut seeds, 3 white testa seed, and 17 purple testa seeds. 'Palkwang' and 'Jakwang' peanut cultivars were examined in three processing methods according to grain, seed coat, and seed. Peanut seeds were processed in two ways, by boiling and by roasting. The former process was as follows: the soil was washed from 1 kg of harvested pods, and the pods were then dried for 2 hours in a shady condition. The pods were boiled for 30 minutes in 4 L water (100℃) and then cooled at room temperature. The latter process consisted of: 2kg of seeds without pod were roasted for 10 minutes in a cauldron (300℃) and then cooled as described above. The contents of *trans*-resveratrol from the two processed peanuts were compared with that of fresh seed without any processing. Peanuts were cultivated according to standard cultural practices in the experimental field of the Honam Agricultural Research Institute. All of the samples were harvested on September 10.

Extraction methods

Prior to analysis, 5g of freeze-dried peanut was homogenized in a blender with 25㎖ of methanol/water (80:20 v/v) and maintained at 30℃ for 45 minutes with gentle stirring. The extract was filtered through nylon membrane filters (0.45㎛) after centrifugation at 8,000 × g for 10 minutes. Solid-phase extraction of *trans*-resveratrol was carried out on C-18 Sep-Pak bonded porous silica cartridges purchased from Waters Associates and pre-conditioned by washing with 5㎖ methanol and 5㎖ distilled water. After passing the sample (5㎖) through the cartridge, the adsorbed resveratrol was eluted with 5㎖ of 80% methanol and 5㎖ of distilled water, and was concentrated in 2㎖ by rotary evaporation.

HPLC analysis

trans-Resveratrol standard (approximately 99%) was purchased from Sigma Chemical Co. A Dionex LC 20 chromatograph and an AD20 absorbance UV-visible detector with 308㎚ was used to detect the *trans*-resveratrol. A Waters Xterra C_{18} column (150 × 4.6 ㎜ I.d., 5㎛ packed column) was used at 40℃ with a flow rate of 1.1㎖/min, and 25㎕ of extract was then injected into the chromatograph. Solvent A was glacial acetic acid in distilled water (52.6:900 v/v) and solvent B was 20% phase A and 80% acetonitrile. The elution profile was as follows: 0 min, 82% A, 18% B 5 min, 82% A, 18% B; 10 min, 70% A, 30% B 15 min, 70% A, 30% B 16 min, 50% A, 50% B 18 min, 0% A, 100% B 20 min, 100% A, 0% B 22 min, 82% A, 18% B.

Results and Discussion

In the *trans*-resveratrol detection of peanuts, the sensitivity of the UV-visible detector system was compared according to the peak area. Fig. 1 shows a typical HPLC chromatogram, in which *trans*-resveratrol was detected at a retention time of 14.5 minutes. Romero-Pérez *et al.* (2001) also detected *trans*-resveratrol at 14.5

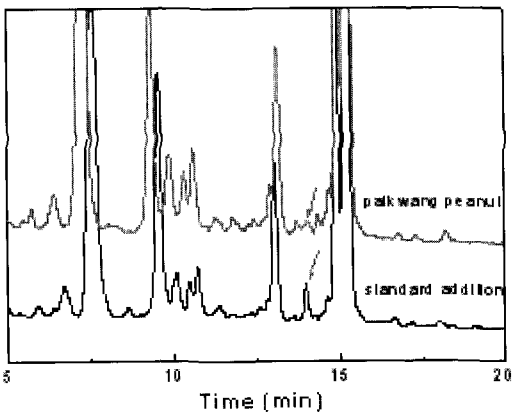

Fig. 1. Typical HPLC chromatogram of the peanut samples with internal standard. Arrows indicate the peaks of *trans*-resveratrol.

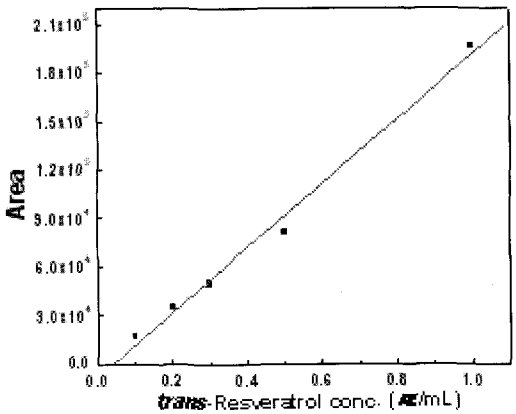

Fig. 2. Calibration curve of *trans*-resveratrol standard solutions. r^2=0.9991, p<0.0001.

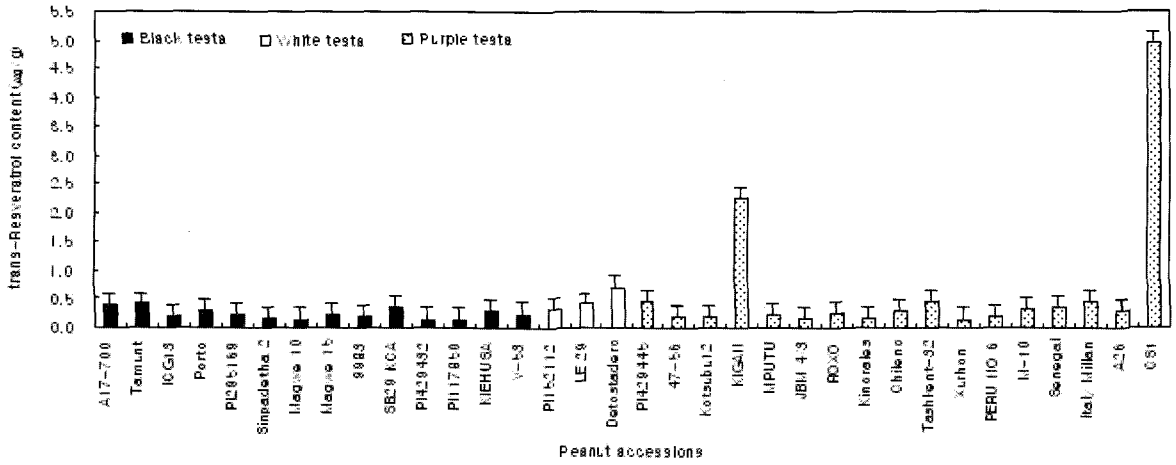

Fig. 3. *trans*-Resveratrol contents in different testa color accessions of peanut.

minutes. Five standards of *trans*-resveratrol covering the range 0.1 ~1.0μg/mℓ were made up in 80% methanol and analyzed in duplicate. These standards were used to evaluate the adequacy of HPLC analysis in terms of its accuracy, the linearity of the calibration curve, and the sensitivity. The constructed calibration curve showed excellent linearity as r^2=0.9991, $p<0.0001$ (Fig. 2).

The *trans*-resveratrol contents various peanut seeds are shown in Fig. 3. Black testa peanut accessions contained 0.14~0.42μg/g *trans*-resveratrol, white peanut accessions contained 0.32~0.72μg /g *trans*-resveratrol, and purple peanut accessions contained 0.14~ 4.96μg/g *trans*-resveratrol. The values obtained were not significantly different based on testa color compared to the 'KIGAN' and 'CS1' accessions, which contained higher *trans*-resveratrol levels (2.26μg/g and 4.96μg/g, respectively) compared to the other seeds.

Based on the processing method used, 'Palkwang' and 'Jakwang' contained 0.36μg/g and 0.22μg/g *trans*-resveratrol, respectively, in fresh peanut accessions, 0.32μg/g and 0.22μg/g in boiled peanut accessions, and 0.40μg/g and 0.26μg/g in roasted peanut accessions, respectively (Fig. 4). Differences among fresh, boiled, and roasted peanuts were not significant. Sobolev and Cole (1999) reported that the quantitation limit of resveratrol in fresh peanuts was approximately 0.01μg/g and roasted peanuts had the lowest contents of resveratrol (0.055±0.023μg/g), while boiled peanuts had the highest resveratrol level, at 5.138±2.849μg/g. In the parts of the seed, the 'Palkwang' and 'Jakwang' grains contained 0.34μg/g and 0.24μg/g *trans*-resveratrol, while testa contained 1.12μg/g and 1.00μg/g, respectively. This result was similar to the report that the contents of resveratrol was higher in testa than in grain (Sanders *et al.*, 2000). However, when comparing absolute quantity, *trans*-resveratrol contents appears to be about 3~ 4 times higher in the testa than in the grain of the peanut, although the total contents did not differ because the testa ratio was low in peanut seeds. In fact, the 100-seed weights of 'Palkwang' and

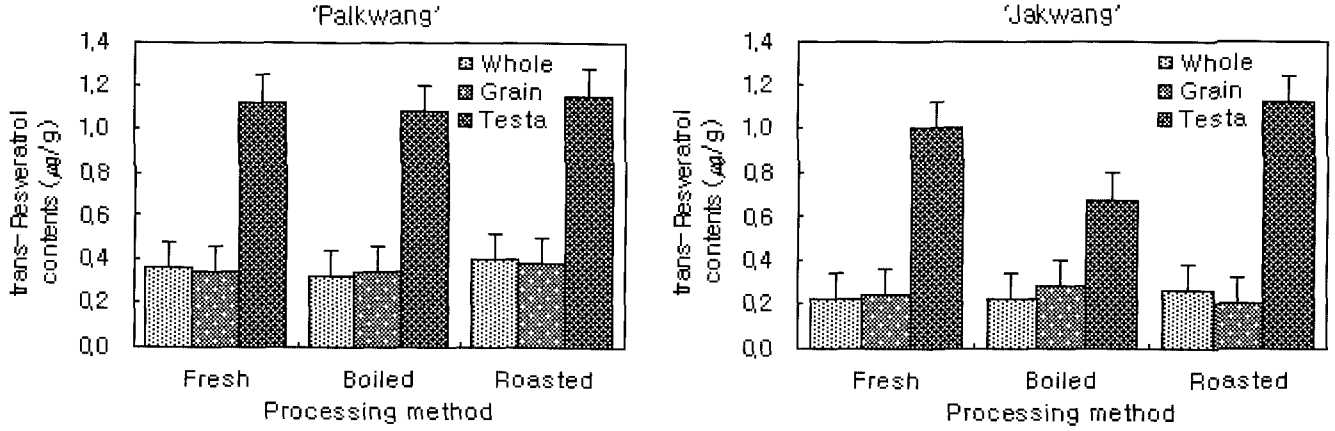

Fig. 4. The contents of *trans*-Resveratrol in peanut by processing method and peanut part.

'Jakwang' are about 88～98g, while the 100-seed testa weights are only 0.4～0.7g. Lee *et al.* (2003) suggested that the testa be used to obtain *trans*-resveratrol because much of the *trans*-resveratrol exists in the testa, even though its contents does not affect the *trans*-resveratrol contents in whole seeds.

However, a big peak was detected before the *trans*-resveratrol peak in the boiled peanut, but was not detected in fresh or roasted peanut (Fig. 5). Considering the area of this peak, we believe that it indicates the presence of more unknown materials than *trans*-resveratrol alone. This material is assumed to be new material that is produced by heat or hydrolysis in the cooking process of peanut seeds.

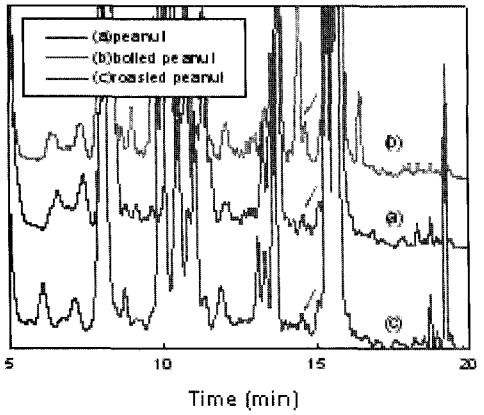

Fig. 5. Typical HPLC chromatogram of the solid phase extracted peanut samples according to processing method (recorded at 308 nm). Arrows indicate the peak of *trans*-resveratrol.

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