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Quantification of *trans*-resveratrol and soyasaponin Bb in different parts of two Korean peanut cultivars

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Abstract Peanut (*Arachis hypogaea* L.) is a legume with edible seeds that can also be processed into oil and butter. Among its bioactive compounds, *trans*-resveratrol and soyasaponin Bb are notable secondary metabolites with potential health benefits. The aim of this study was to quantify these metabolites in various parts of two Korean peanut cultivars, 'Sinpalkwang' and 'Sewon,' by using reverse-phase high-performance liquid chromatography equipped with photodiode array and evaporative light-scattering detectors. Both cultivars exhibited the highest *trans*-resveratrol content in the cotyledons (0.035 mg/g extract in 'Sinpalkwang' and 0.027 mg/g extract in 'Sewon') and the highest soyasaponin Bb content in the roots (21.507 mg/g extract in 'Sinpalkwang' and 28.957 mg/g extract in 'Sewon'). Moreover, the amounts of secondary metabolites differed depending on the cultivar and phytotoxin classification. These findings provide valuable insights into the distribution of secondary metabolites in various peanut plant parts and contribute to our understanding of the bioactive compound variation in Korean peanut cultivars.

Keywords High-performance liquid chromatography · Peanut · Sewon · Sinpalkwang · Soyasaponin Bb · *trans*-Resveratrol

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Introduction

Peanut (*Arachis hypogaea* L.), also called goober, groundnut, or earthnut, is a dicotyledonous plant belonging to the Leguminosae family used in various foodstuffs such as snacks, oils, and butter. Peanuts not only contain protein, carbohydrates, vitamins, and minerals but also healthy unsaturated fatty acids such as oleic acid and linoleic acid [1]. The increasing consumption of peanuts has led to the development of 'Sinpalkwang' (SPK) and 'Sewon' (SW) peanut cultivars at the Department of Southern Area Crop Science, National Institute of Crop Science.

The SPK cultivar, a "Virginia" type with many branches and high yield, contains 29.2% crude protein and 45.4% crude fat, while its unsaturated fatty acid composition includes 43.9% oleic acid and 41.5% linoleic acid [2]. The SW cultivar, a "Shinpung" type with a small number of branches, is an early maturing variety, with its low-fat green peanuts being harvested 20 days before maturity. It contains 31.7% crude protein and 35.2% crude fat, while its unsaturated fatty acid composition includes 52.3% oleic acid and 25.3% linoleic acid [3]. They are large-seed varieties that require a high accumulative temperature of over 3300 ºC and have shown high adaptability in the southern region of Korea. Since SPK and SW were selected as new varieties in 2012 and 2017, respectively, they have been widely cultivated in Korean farms due to their high yield and are still highly preferred by domestic consumers [2,3].

In the constantly changing external environment, plants can experience biotic or abiotic stress that is overcome by activating their defense system. The latter comprises a constructive and inducible system to mitigate environmental stress and plant diseases. Phytotoxins, which are secondary metabolites produced by plants for self-defense, are classified into phytoalexins and phytoanticipins; the latter, including saponins, are stored in the plant without external stimulation [4]. Soyasaponin, which is toxic at high concentrations and has antifeedant and antiherbivore effects, protects plants from predators. It also has a bitter taste and reduces the digestive power of ruminants [5]. Moreover, soyasaponin derivatives have

been reported to have anti-tumor and hepatoprotective effects [6]. Phytoalexins, including stilbenes such as resveratrol, protect plants from invasion by external pathogenic bacteria. [7]. Stress induced by UV radiation, ozone, infection, and chemicals promotes the synthesis of resveratrol, which accumulates in the plant [8], an activity that has been commercially exploited [9]. *trans*-Resveratrol, which is more stable than the *cis*-form, not only has pharmaceutical properties that prevent cardiovascular disease and regulate serum glucose levels but also has a protective role that extends the shelf life of fruits [10,11]. Although many studies have been conducted on *trans*-resveratrol, soyasaponin Bb (the most basic form of soyasaponin) is still unexplored [12].

In the present study, the amounts of *trans*-resveratrol and soyasaponin Bb in various tissues (roots, cotyledons, hypocotyls, and epicotyl) of two Korean peanut cultivars, SPK and SW, were analyzed by using reverse-phase high-performance liquid chromatography (HPLC) with photodiode array (PDA) and evaporative light-scattering (ELS) detectors. To achieve more precise measurements, we sought the optimal detector for each of the compounds.

Materials and Methods

Plant materials

SPK and SW were provided by Jeonbuk State Agricultural Research, Iksan, Korea (Fig. 1). Voucher specimens were stored in Jeonbuk State Agricultural Research, Iksan, Korea.

Growth conditions

The SPK and SW plant materials (root, cotyledon, hypocotyl, and epicotyl) were supplied by Jeonbuk State Agricultural Research, Iksan, Korea. On 1st September 2024, SPK and SW peanut seeds were soaked in water for 24 h, then transferred to a cultivation tray and cultivated aeroponically. The spray system was programmed to spray water for 30 s at 10 m intervals. The aeroponic process continued until harvesting on 10th September 2024.

Instruments and reagents

The plant materials were analyzed using reverse-phase HPLC (Waters Alliance system e2695 Separations Module, Milford, MA, USA) with 2998 PDA and 2424 ELS detectors (Waters Corporation, Milford, MA, USA). HPLC-grade water (H_2O) and methanol (MeOH) were purchased from Honeywell (Burdick and Jackson, Muskegon, MI, USA). HPLC-grade acetic acid and phosphoric acid were purchased from Fisher Scientific (Loughborough, Leicestershire, UK). HPLC-grade trifluoroacetic acid (TFA) was obtained from Thermo Fisher Scientific (Ward Hill, MA, USA). HPLC-grade acetonitrile (ACN) was obtained from J. T. Baker (Phillipsburg, PA, USA). Ethanol (EtOH) was purchased from Samchun Chemicals (Pyeongtaek, Korea). *trans*-Resveratrol and soyasaponin Bb were provided by the Natural Product Institute of Science and Technology (www.nist.re.kr) (Anseong, Korea) (Fig. 2).

Preparation of the standard and sample solutions

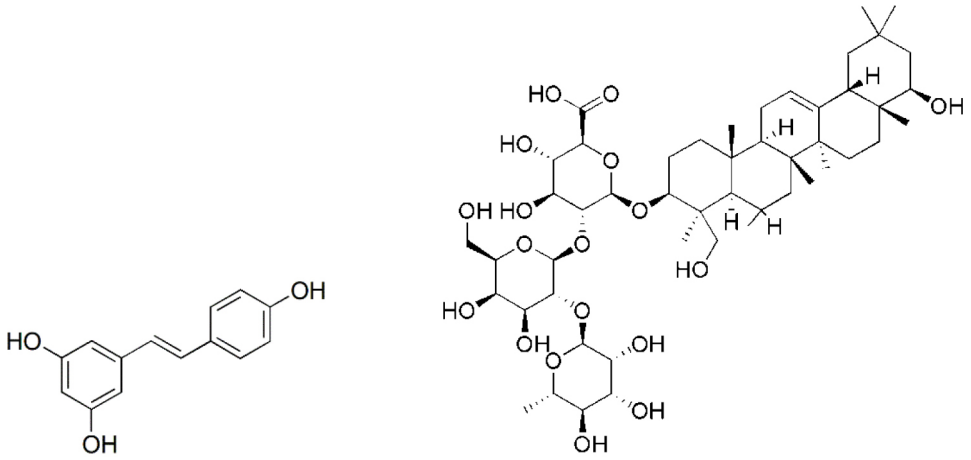
The roots, cotyledons, hypocotyls, and epicotyls of 'SPK' and 'SW' were extracted using 95.0% EtOH using a reflux condenser (Table 1). The solution extracted from the sample was filtered to remove impurities, after which the solvent was completely removed using a rotary evaporator. The extracts (50 mg) and the standard were dissolved in 80% MeOH, after which the solutions were filtered through a polyvinylidene fluoride membrane filter with a pore size of 0.45 μm (Hyundai Micro, Seoul, Korea).

HPLC-PDA conditions for *trans*-resveratrol measurements

The analysis of *trans*-resveratrol was progressed by using HPLC-PDA equipped with a reverse-phase column (INNO C_{18} column, 4.6×250 nm, Youngjin Biochrom, Seongnam, Korea) at 35 °C. The standard (1,000 ppm; 1 mg/mL) and sample (50,000 ppm; 50 mg/mL) solutions were sonicated for 20 min to ensure even mixing and then filtered. The injection volume was 10 μL and the detection wavelength was 310 nm. Mobile phases A and B were water containing 0.5% acetic acid and ACN, respectively. The flow rate was 1.0 mL/min. The A/B ratio was varied as 85:15 for 5 min, 77:23 for 10 min, and then 70:30 for the remainder of the process.

(A) (B)

Fig. 1 Photographic images of SPK (A) and SW (B). 1, the roots; 2, the hypocotyl; 3, the cotyledon; 4, the epicotyl including the leaves



trans-resveratrol soyasaponin Bb

Fig. 2 Chemical structures of *trans*-resveratrol and soyasaponin Bb

Table 1 Details of the SPK and SW samples

Sample	Dry Weight (g)	Extract Weight (g)	Yield (%)
SPK 1	1.5	0.6	40.0
SPK 2	2.3	1.3	56.5
SPK 3	2.3	1.2	52.2
SPK 4	2.3	1.1	47.8
SW 1	1.6	0.5	31.3
SW 2	2.7	1.5	55.6
SW 3	4.4	2.1	47.7
SW 4	2.1	0.9	42.9

The number suffixes refer to different parts of the plants: 1, the roots; 2, the hypocotyl; 3, the cotyledon; 4, the epicotyl including the leaves

Afterward, the column was washed with ACN 100 for 15 min, after which the A/B ratio was set to 85 : 15 for 10 min to stabilize it.

HPLC-ELSD conditions for soyasaponin Bb measurements

Analysis of soyasaponin Bb content was conducted by using HPLC-ELSD with a reverse-phase INNO C_{18} column at 30 ºC. The sample and standard solutions were made at the same concentration (2000 ppm; 2 mg/mL). The injection volume was 10 µL and the flow rate was set at 1 mL/min. Mobile phases A and B were water containing 0.1% TFA and ACN, respectively. ELSD was conducted at a drift tube temperature of 70 ºC with the gain set at five, and nitrogen gas at a pressure of 50.0 psi was used as the spraying gas. As per the method of Lin and Wang [13], the A content was 70% for 5 min, then decreased to 60% at 5 min, 52% at 40 min, 50% at 41 min, 0% from 42 to 44 min, and 70% at 45 min for 15 min.

Method validation

The HPLC method used to quantify *trans*-resveratrol and soyasaponin Bb in SPK and SW was validated for limit of detection (LOD) and limit of quantification (LOQ). The peak areas (y) of the five concentrations of standards (x) used to draw the calibration curve were measured three times. LOD and LOQ were calculated using the calibration curve's slope (S) and the calibration curve intercept's standard deviation values (σ).

$$\text{LOD} = 3.3 \times \frac{\sigma}{S}$$

$$\text{LOQ} = 10 \times \frac{\sigma}{S}$$

Calibration curve generation

Standard solutions at 5 different concentrations (0.97, 1.95, 3.90, 7.81, and 15.63 ppm) were measured, after which a calibration curve of *trans*-resveratrol was plotted with the x-axis as the concentration and the y-axis as the peak area. The calibration equation used was $y = 83536x - 7168.3$ with a coefficient of determination (r^2) of 0.9998. The standard solutions for soyasaponin Bb were 125, 250, 500, 1000, and 2000 ppm. Because the ELSD results were non-linear, the concentration of the standard (x) and peak area of HPLC chromatography (y) used to draw the calibration curve were log-transformed values. The calibration curve equation was $y = 1.5608x - 0.9155$ with $r^2 = 0.9929$.

Statistical analysis

All of the experiments were conducted three times, and the results are expressed as the mean ± standard deviation. Significant differences between samples were determined using analysis of variance (ANOVA) and Tukey's *post hoc* tests with the significance

level set at p <0.05 using Minitab 16 (Minitab Inc., State College, PA USA).

Results and Discussion

Using the knowledge that *trans*-resveratrol has the highest UV absorbance at approximately 310 nm, the peak for the *trans*-resveratrol standard solution was observed at an elution time of 25.8 min. The peaks of the UV spectra of the *trans*-resveratrol standard and sample were detected at the same wavelength and retention time (Fig. 3). Thereby, the content *trans*-resveratrol in the samples was calculated using the calibration equation (Table 2). As expected, only very small concentrations of *trans*-resveratrol were present in peanut tissues in the absence of infection [14]. Among the SPK samples, the sample with the highest *trans*-resveratrol content was SPK 3 (0.035 mg/g), and the sample with the lowest content was SPK 4 (0.003 mg/g). Among the SW samples, the sample with the highest *trans*-resveratrol content was SW 3 (0.027 mg/g), and the sample with the lowest content was SW 4 (0.007 mg/g) (Table 3). Thus, both cultivars showed the highest content in the cotyledon and the lowest content in the epicotyl (Fig. 4). Wang et al. [14,15]

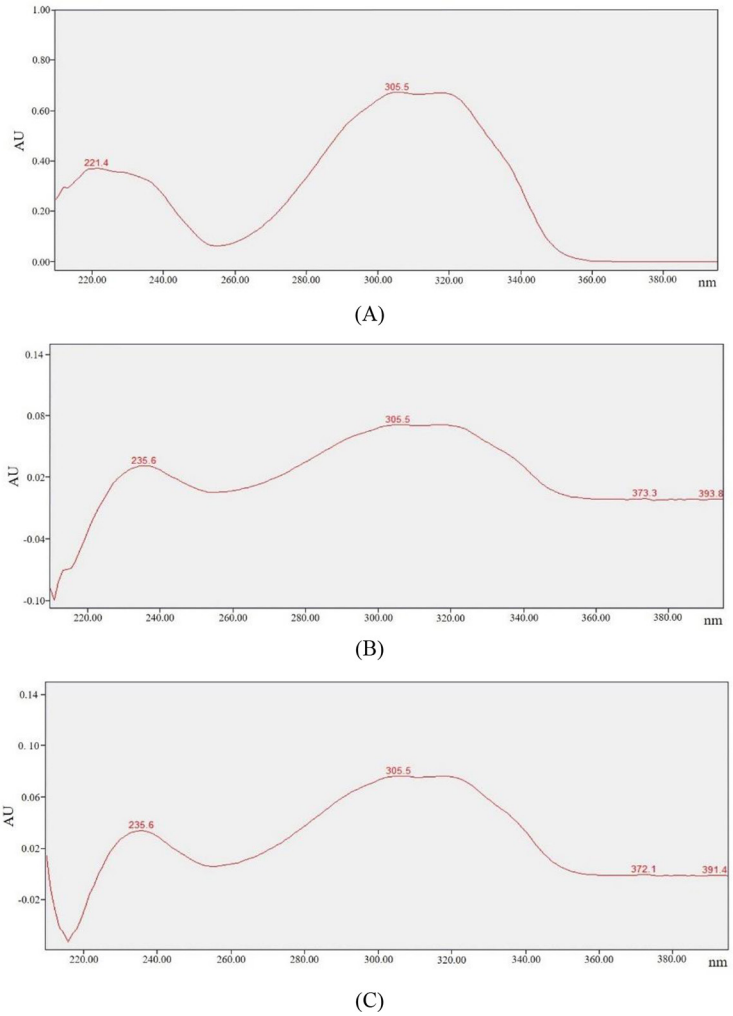


Fig. 3 UV spectra for *trans*-resveratrol (A), SPK (B) and SW (C)

Table 2 Calibration curve details

Compound	t_R (min)	Range (µg/mL)	Calibration equation	r^2	LOD (µg/mL)	LOQ (µg/mL)
trans-Resveratrol	25.8	0.97 – 15.63	$y = 83536\,x - 7168.3$	0.9998	0.0490	0.1486
Soyasaponin Bb	17.4	12.5 – 2000	$y = 1.5608\,x - 0.9155$	0.9929	0.2787	0.8446

x, the concentration of the standard (µg/mL); y, peak area; r^2 = coefficient of determination based on three data points in the calibration curve; t_R, retention time in the HPLC chromatograph; LOD, limit of detection; LOQ, limit of quantification

Table 3 Amounts of *trans*-resveratrol and soyasaponin Bb in the SPK and SW samples

Sample	Amount (mg/g ext.)		
	trans-Resveratrol	Soyasaponin Bb	Total
SPK 1	0.012±0.000[b]	21.507±0.794[a]	21.519
SPK 2	0.013±0.000[b]	6.335±0.129[c]	6.348
SPK 3	0.035±0.001[a]	1.109±0.124[d]	1.144
SPK 4	0.003±0.000[c]	8.530±0.307[b]	8.533
SW 1	0.017±0.000[b]	28.957±0.663[a]	28.974
SW 2	0.010±0.000[c]	8.509±0.549[c]	8.519
SW 3	0.027±0.000[a]	2.064±0.171[d]	2.091
SW 4	0.007±0.001[d]	13.812±1.038[b]	13.819

[a-d]Different letters in the same column for the same compound indicate statistically significant differences between the values (p <0.05). The number suffixes refer to different parts of the plants: 1, the roots; 2, the hypocotyl; 3, the cotyledon; 4, the epicotyl including the leaves

reported that the *trans*-resveratrol content in cotyledons and was higher than that in the roots of peanut cultivars in Taiwan. This is the same result as in the present study, where SPK 3 and SW 3 had the highest *trans*-resveratrol content. The peak found at the same retention time as that of standard *trans*-resveratrol is indicated by an arrow (Fig. 5).

The *trans*-resveratrol content was generally low in all samples. This is unsurprising because it is a phytoalexin synthesized in response to an attack from an external pathogen. For instance, when comparing the *trans*-resveratrol content in extracts from infected and uninfected grape (*Vitis vinifera* L.) leaves, more *trans*-resveratrol was detected in the pathogen-infected leaves [16]. Other factors that can affect *trans*-resveratrol content are pH, sunlight, temperature, and oxidation [17-19]. However, in this experiment, the possibility of changes in *trans*-resveratrol content due to infection, pH, sunlight, and oxidation is very low. The peanuts in the present study were grown in water with a pH of approximately 6 at an average temperature of 25 °C. Since *trans*-resveratrol is stable for up to 28 days in the pH range of 1 to 7 [17], it is unlikely that the pH used in our study reduced the *trans*-resveratrol content. Although sunlight (especially in the UV range) can change *trans*-resveratrol into other compounds [17,18], it is unlikely that the fluorescent lights in our

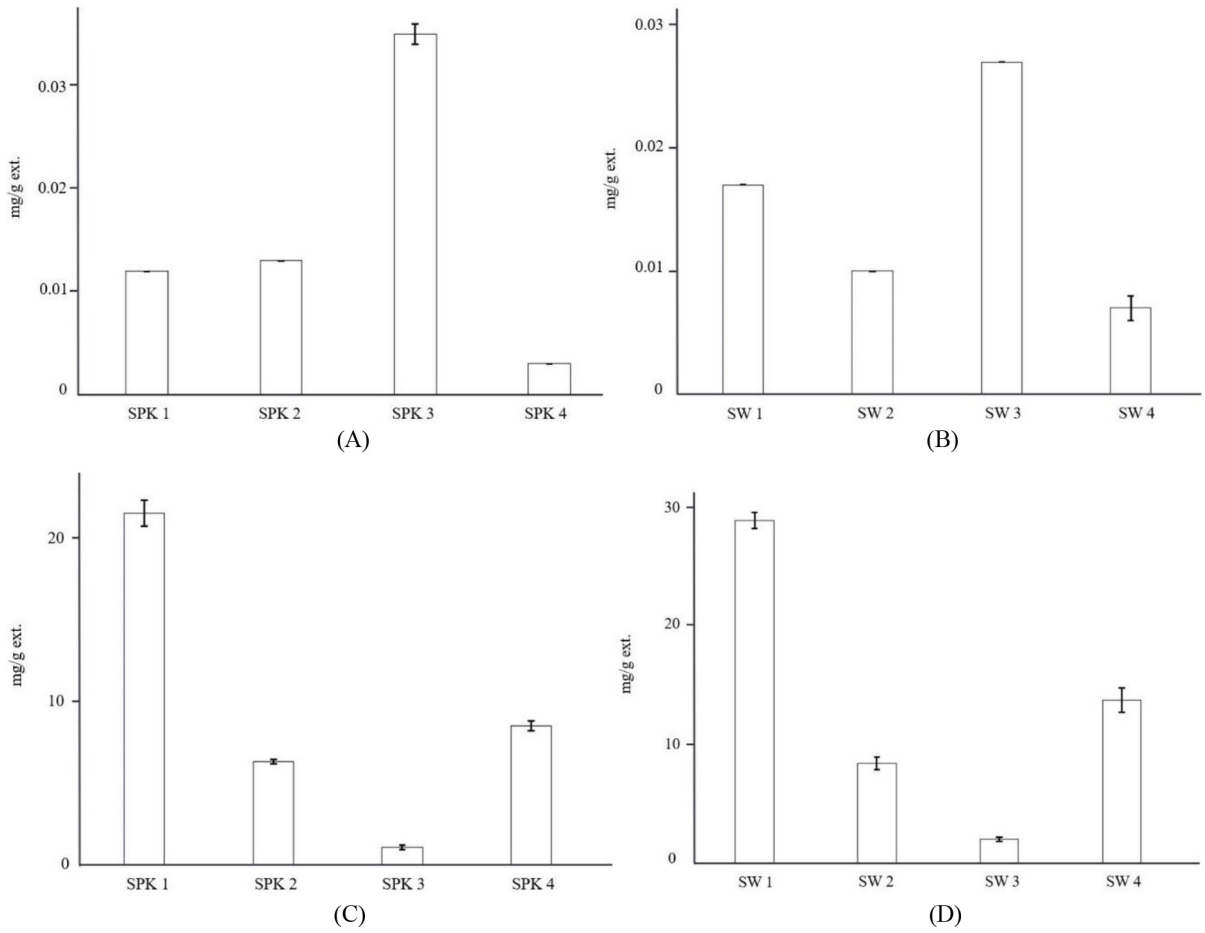


Fig. 4 Bar charts showing the *trans*-resveratrol content in SPK (A) and SW (B) and the soyasaponin Bb content in SPK (C) and SW (D). The number suffixes refer to different parts of the plants: 1, the roots; 2, the hypocotyl; 3, the cotyledon; 4, the epicotyl including the leaves

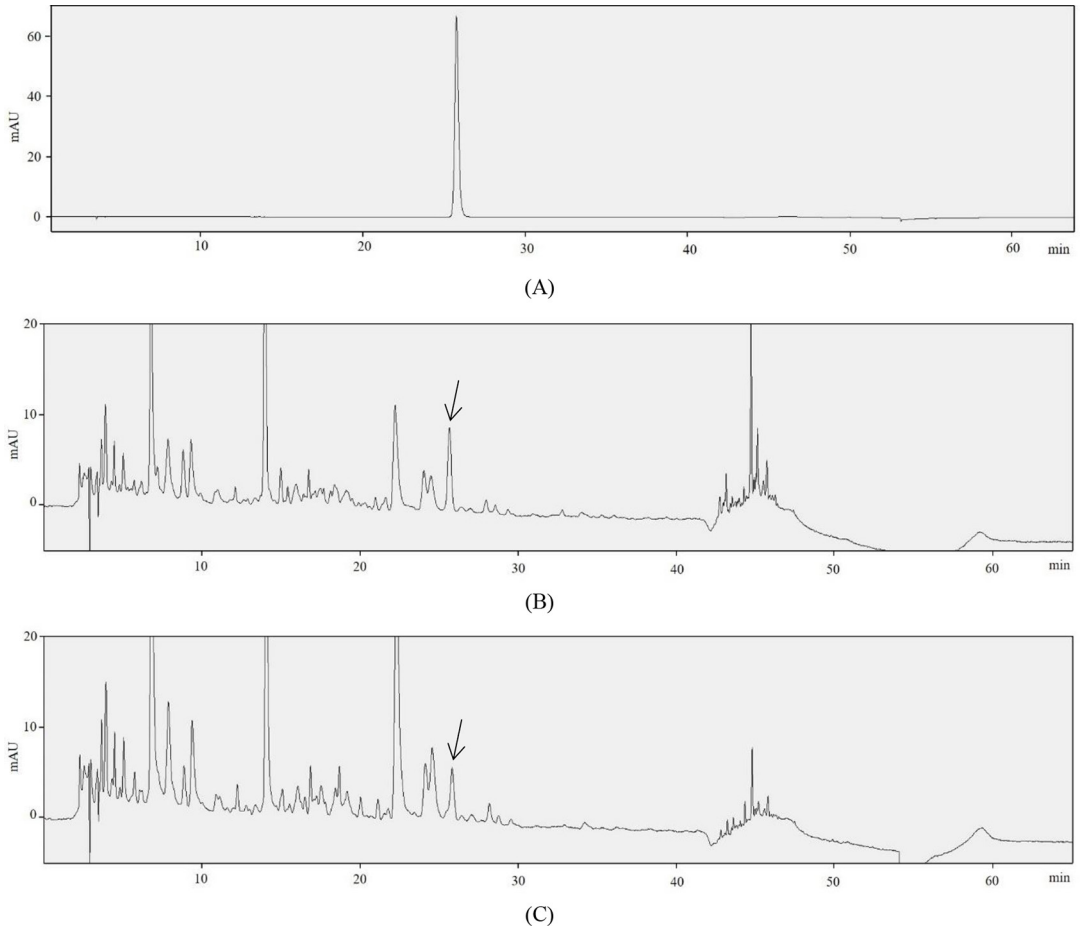


Fig. 5 HPLC/PDA chromatograms for *trans*-resveratrol (A) in SPK 3 (B) and SW 3 (C). The arrow indicates the peak of *trans*-resveratrol in the sample

laboratory could induce transformation. Oxidation is not applicable because it requires other metal constituents [19]. The *trans*-resveratrol content could have been changed by the temperature condition. In another study, heating wine at 30 ºC for 24 h reduced the *trans*-resveratrol content by approximately half while the δ-viniferin content (a derivative of *trans*-resveratrol) increased five-fold [19]. In the present study, *trans*-resveratrol was extracted three times for 3 h at approximately 60 ºC, this temperature condition could have changed it into a different form.

Since soyasaponin Bb does not have sufficient chromophoric groups in its molecular structure, its content had to be measured at a low wavelength (below 210 nm) using HPLC-PDA. In a preliminary experiment, it was found that HPLC-ELSD yielded a higher content than HPLC-PDA in the same sample. Therefore, the soyasaponin Bb content was analyzed using the former method. The standard peak of soyasaponin Bb was detected at 17.4 min in the elution chromatogram. A spike test was used to confirm whether the peak for soyasaponin Bb was correct. A spike test involves adding a small amount of standard to a sample and estimating that the same substance as in the standard exists in the sample if the HPLC peak area increases [20]. Among the four parts of SPK, the sample with the highest soyasaponin Bb content was SPK 1 (21.507 mg/g) and the sample with the lowest content was SPK 3 (1.109 mg/g). Among the SW samples, the sample with the highest soyasaponin Bb content was SW 1 (28.957 mg/g), and the sample with the lowest content was SW 3 (2.064 mg/g) (Table 3). Thus, the highest soyasaponin Bb content was found in the roots of both cultivars. The peak found at the same retention time as the peak of standard soyasaponin Bb is indicated by an arrow (Fig. 6).

Soyasaponin Bb is an exudate secreted in roots that regulates plant growth at low concentrations and acts as a growth inhibitor at high concentrations. Found predominantly in root exudates of soybean, alfalfa, pea, and peanut plants [22], it is known to prevent the germination of plants in which the roots have been parasitized [21]. Nevertheless, soyasaponin Bb is widely distributed throughout the plant. It is amphipathic due to the hydrophilic sugar chain and hydrophobic aglycone (soyasapogenol B) in its structure that enables it to permeate cells, thereby enabling it to move to other parts of the plant or accumulate in the root as an exudate [5,21]. In our study, both cultivars showed the same trend, with the soyasaponin

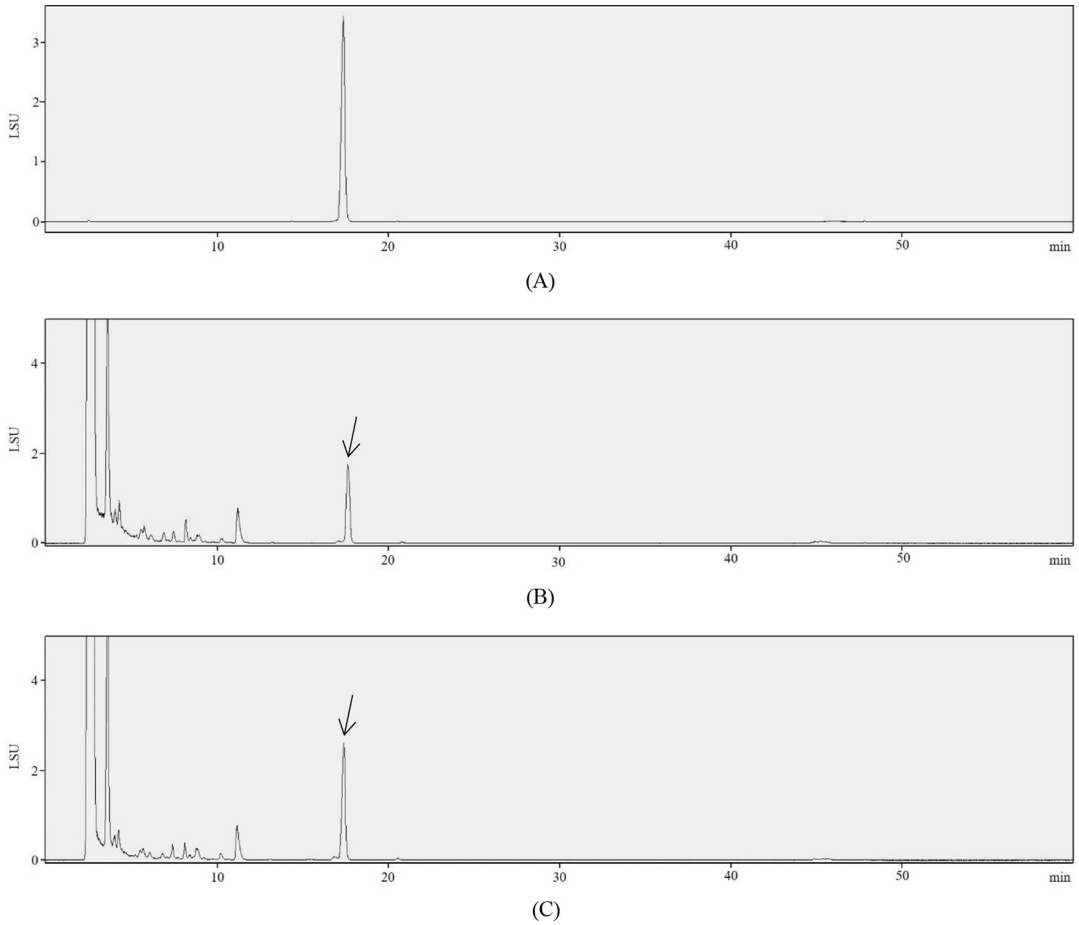


Fig. 6 HPLC/ELSD chromatograms for soyasaponin Bb (A) in SPK 1 (B) and SW 1 (C). The arrow indicates the peak of soyasaponin Bb in the sample

Bb content decreasing in the order of the epicotyl, hypocotyl, and cotyledon (Fig. 4). The high soyasaponin Bb content in the epicotyl appears to be due to a large area of the leaves being exposed to light, which changed its content by regulating the expression of genes involved in its synthesis [23].

In this study, we analyzed the amounts of *trans*-resveratrol and soyasaponin Bb in four different parts of the peanut plant (root, hypocotyl, cotyledon, and epicotyl) using reverse-phase HPLC and a different detector for each compound. The results of the analysis show that both peanut cultivars had the highest *trans*-resveratrol content in the cotyledons and that the total *trans*-resveratrol content was slightly higher in SPK (0.063 mg/g) than in SW (0.061 mg/g). Soyasaponin Bb was found to be very high in the roots of both cultivars, and the content was higher in SW (53.342 mg/g) than in SPK (37.481 mg/g).

The *trans*-resveratrol content of samples collected from healthy plants did not differ significantly between the two cultivars. However, the soyasaponin Bb content, which is produced and accumulates without external stimulation, varies depending on the cultivar. Although we only compared two cultivars, the levels of secondary metabolites, especially phytoanticipins, can be different between two cultivars grown in the same environment. Simply changing the cultivar may enable obtaining more of the targeted compounds.

trans-Resveratrol, which is abundant in cotyledons, participates in the regulation of tumor cell activity from initiation to termination, thereby exerting an anticancer effect, and can also be consumed as a dietary supplement to prevent chronic diseases related to aging [24,25]. It is used not only in medicine but also in agriculture: for instance, as an insecticide to repel pests or to reduce the generation of ethylene, which promotes ripening during the storage of agricultural products. It can be used for pesticides and post-harvest treatments [24]. Since soyasaponin Bb can regulate water-electrolyte metabolism and blood pressure, it is effective in treating hypertension. It also has the effect of reducing weight by blocking the intestinal absorption of fat. Although further research is needed, soyasaponin Bb, which is abundant in the roots of SPK and SW, has the potential to be used as an anticancer, antidiabetic, and antiobesity supplement or therapeutic agent [26,27]. Although *trans*-resveratrol has already been studied extensively enough for use as a dietary

supplement, that is not the case for soyasaponin Bb, so its potential is largely unknown.

This study's findings provide valuable insights into the distribution of secondary metabolites in various peanut plant parts and improve our understanding of the bioactive compound variation in Korean peanut cultivars.

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