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# Comparison of Isoflavones and Anthocyanins in Soybean [*Glycine max* (L.) Merrill] Seeds of Different Planting Dates

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**ABSTRACT:** The concentrations of isoflavones and anthocyanins in five soybean seed cultivars of three different planting dates were determined in this study. Among the seeds of three different planting dates, the highest concentration of average total isoflavones (4098 $\mu g\ g^{-1}$) was measured in those from early July, whereas the lowest concentration of average total isoflavones (3238 $\mu g\ g^{-1}$) was measured in those from late May. Anthocyanin compounds were detected only in the Cheongjakong 3 cultivar. Among the three different planting dates, late-planted Cheongjakong 3 accumulated the highest concentration of total anthocyanins (10103 $\mu g\ g^{-1}$), whereas the variety at an earlier planting date exhibited the lowest concentration of average total anthocyanins (7115 $\mu g\ g^{-1}$). On the basis of these results, it was concluded that environmental factors such as temperature and precipitation may change the isoflavone and anthocyanin contents of soybean, altering the nutritional values of soy products.

**KEYWORDS:** *soybean, planting date, isoflavones, anthocyanins*

## INTRODUCTION

Soybean is a well-known staple food that contains starch, dietary fiber, protein, lipids, and essential minerals as well as beneficial secondary metabolites such as isoflavones, phenolic compounds, and soyasaponins.[1] In addition, soybeans possess significant amounts of phenolic and polyphenolic compounds, including flavonoids, phenolic acids, lignins, and soyasaponins.[1] These compounds have antioxidant properties, which have beneficial effects on human health.[2]

Soybean is a valuable and popular crop globally, especially in Asia, and is used to produce a variety of products such as soy paste, soybean sprouts, soy curd, soy milk, tofu, and oil. Notably, soy has protective effects against heart disease, osteoporosis, and various types of cancers.[3] For example, soybean ingestion is associated with a decreased risk of cardiovascular disease, osteoporosis, and cancer (including breast and colon cancers).[2]

Isoflavones and anthocyanins, both of which are beneficial for human health, are found in soybeans.[4] Isoflavones are the most common form of phytoestrogens found in various plants, including legumes, seeds.

Anthocyanins are secondary metabolites and are water-soluble pigments, which are responsible for the red, purple, and blue coloration of many fruits, vegetables, and cereal grains. They belong to a widespread class of phenolic compounds.[5] In plants, anthocyanins play roles in attracting animals for pollination or seed propagation, plant-specific flowering, antibacterial activities, protecting cells from UV radiation, and producing phytoalexin for signal transmission. Anthocyanins have beneficial effects on the human body, including antioxidant and anticancer activities, lowering blood cholesterol, preventing heart disease, and protection from UV radiation.[6] As a comparison, anthocyanins have greater antioxidant activity than both vitamins E and C.[7,8]

Anthocyanins include cyanidin, cyanidin-3-glucoside, delphinidin, malvidin, malvidin-3-glucoside, pelargonidin, pelargonidin-3-glucoside, peonidin, peonidin-3-glucoside, and petunidin.[9] Additionally, several major anthocyanins (cyanidin-3-glucoside, delphinidin-3-glucoside, and petunidin-3-glucoside) have been isolated from the seed coat of black soybeans.[10,11] Black soybeans are widely used as a health food and folk medicine in China, Taiwan, Japan, and Korea.[11]

In this study, five soybean seeds were cultivated in the Yeoju region (Yeojugun, Gyeonggi-Do, Korea) at three different planting dates. In a previous study, soybean seeds grown in different regions or years or sown at different times under the same field conditions within the same year showed variations in their isoflavone profiles. Isoflavone contents and their distribution in soybeans fluctuate in response to various factors such as location, crop year, growth condition, temperature, soil nutrition, and duration of storage.[12,13] Consistent with this finding, our previous work demonstrated that the isoflavone content of soybean seeds varies according to environmental factors such as location and years of storage.[12] An earlier study also reported that isoflavone composition is affected by the soybean genotype.[14]

The objective of this study was to determine the effects of different planting dates on the isoflavone and anthocyanin contents in the soybean seeds of five cultivars grown in Yeoju in 2011. The results of this study provide further insight into the influence of environmental factors on the isoflavone and anthocyanin contents in soybean seeds.

## MATERIALS AND METHODS

**Preparation of Soybean Seeds.** Five varieties of soybean seeds (Cheongjakong 3, Somyungkong, Daewonkong, Taekwangkong, and Hwanggeumkong) were cultivated on Konkuk University's farm in the

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**Table 1. Calibration Curve Equations of the 12 Isoflavone Standards**

| isoflavone | equation | $r^2$ |
|---|---|---|
| daidzin | $y = 57249x - 228408$ | 0.999 |
| glycitin | $y = 49678x - 131686$ | 0.999 |
| genistin | $y = 94070x - 344977$ | 0.999 |
| malonyldaidzin | $y = 51974x - 434646$ | 0.996 |
| malonylglycitin | $y = 49232x - 333612$ | 0.997 |
| malonylgenistin | $y = 77677x - 605833$ | 0.996 |
| acetyldaidzin | $y = 60476x - 340235$ | 0.997 |
| acetylglycitin | $y = 59012x - 228910$ | 0.999 |
| acetylgenistin | $y = 82361x - 174691$ | 0.999 |
| daidzein | $y = 114755x - 78738$ | 0.999 |
| glycitein | $y = 98693x - 9111.8$ | 0.999 |
| genistein | $y = 136656x + 68589$ | 0.999 |

**Table 2. Calibration Curve Equations of the Nine Anthocyanin Standards**

| anthocyanin | equation | $r^2$ |
|---|---|---|
| cyanidin-3-glucoside | $y = 72.84x - 2857.3$ | 0.999 |
| pelargonidin-3-glucoside | $y = 44.743x - 1235$ | 0.999 |
| peonidin-3-glucoside | $y = 28.257x - 798.14$ | 0.999 |
| malvidin-3-glucoside | $y = 68.053x - 2400.4$ | 0.999 |
| delphinidin | $y = 68.8x - 99.51$ | 0.999 |
| cyanidin | $y = 74.233x - 852.33$ | 0.999 |
| pelargonidin | $y = 60.456x - 605.08$ | 0.999 |
| peonidin | $y = 88.765x - 1405.9$ | 0.999 |
| malvidin | $y = 19.538x - 200.23$ | 0.999 |

Yeoju region. All of the soybean seed varieties were cultivated during the mid-to-late season. Generally, they are sown in late June and harvested in early October in the central districts of Korea. The experimental and climate conditions as well as plot management applied were the same as those described by Lee et al.[12] Field experiments were conducted in 2011 to determine the effects of three different planting dates (May 25, June 21, and July 10) on the concentrations of soybean isoflavones and anthocyanins. Each variety of soybean was harvested August 31 (May 25 planting), September 28 (June 21 planting), and October 18 (July 10 planting). Fertilizer was applied prior to planting at the following recommended rates: 80, 80, and 120 kg $ha^{-1}$ for N, $P_2O_5$, and $K_2O$, respectively. The preceding crop produced soybeans at all sites. Thus, rhizobial inoculation was not necessary. Planting was performed at a density of 50 seeds $m^{-2}$ with plants thinned to a uniform density 14 days after planting. Each plot consisted of three rows (3.75 m in length and 0.6 m between rows), and plots at each site were assigned to a completely randomized design with two replicates. Planting dates were randomly assigned to each variety of soybean seed, which were harvested from each replicate at each site. After the seeds were harvested, they were freeze-dried, ground, and stored at room temperature until further analysis.

**Analysis of Isoflavones in Soybean Seeds.** Each sample of the five soybean varieties (Cheongjakong 3, Somyungkong, Daewonkong, Taekwangkong, and Hwanggeumkong) was dried in a freeze-dryer (FreeZone 4.5; Labconco, Kansas, MO, USA) under vacuum and then ground. For this experiment, methanol (100%), acetonitrile (100%), glacial acetic acid (99.9%), and distilled water were purchased from J. T. Baker [Phillipsburg, NJ, USA; high-performance liquid chromatography (HPLC) grade), and hydrochloric acid (HCl) was purchased from Daejung Co. (Daejung Chemical & Materials Co. Ltd., Siheung, Gyeonggi-Do, Korea). The extraction of isoflavones from soybean samples was performed according to the method described by Wang and Murphy.[4] Two replications of extractions from each sample of soybean seeds were performed. The extraction solvent was composed of 10 mL of acetonitrile and 2 mL of 0.1 N HCl per sample, and ground soybean samples (2 g) were extracted using the extraction solvent followed by stirring for 2 h at room temperature (Green-Sseriker; Vision Scientific Co. Ltd., Bucheon, Gyeonggi-Do, Korea). The extract was filtered through a no. 42 Whatman filter paper (100 circles, 125 mm in diameter; Maidstone, UK) and concentrated using a vacuum evaporator (EYELA; Tokyo Rikakikai, Co. Ltd., Japan) at a temperature lower than 40 °C. The residues were redissolved in 10 mL of 100% aqueous methanol (HPLC grade; J. T. Baker), filtered through a 0.2 $\mu$m nylon membrane syringe filter (17 mm, Titan; Sunsri, Rockwood, TN, USA), and transferred into a 2-mL vial and analyzed by HPLC.

HPLC analysis was performed according to the modified method described by Lee et al.[12] A Younglin YL 9100 HPLC system equipped with a YL 9120 UV−vis detector, YMC ODS AM-303 YL 9150 autosampler, and a 5 $\mu$m (250 × 4.6 mm) i.d. column was used. The wavelength of the UV detector was set to 254 nm. The mobile phase consisted of 0.1% glacial acetic acid in distilled water (solvent A) and 0.1% glacial acetic acid in actontrile (ACN) (solvent B). Solvent B was increased from 15 to 35% for 50 min and then held at 35% for 10 min. The solvent flow rate was 1 mL $min^{-1}$, and the injection volume was 20 $\mu$L per sample.

Standards for 12 isoflavones (daidzein, genistein, glycitein, daidzin, genistin, glycitin, acetyl daidzin, acetylgenistin, acetylglycitin, malonyldaidzin, malonylgenistin, and malonylglycitin) were purchased from LC Laboratories (Woburn, MA, USA). The isoflavones standards were dissolved in dimethyl sulfoxide (DMSO; Sigma-Aldrich, USA) at several concentrations (25, 50, 100, and 150 $\mu$g $mL^{-1}$), and a high linearity ($r^2 > 0.996$) was obtained for each compound (Table 1). The 12 isoflavones were identified by their retention times, and their concentrations were calculated by comparing the peak areas of samples with those of the standards.

**Analysis of Anthocyanins in Soybean Seeds.** The extraction of anthocyanin from soybean Cheongjakong 3 variety was performed according to the modified method of Kim et al.[8] Ground soybean (0.2 g; Choengjakong 3) was mixed with 2 mL of 1% HCl in 80% MeOH for 24 h at 4 °C in dark conditions. After centrifugation at 13000 rpm for 10 min, each specimen was filtered through a 0.2 $\mu$m nylon membrane syringe filter (17 mm, Titan; Sunsri) and analyzed by HPLC.

The linear gradient reported by Kim et al.[8] and Sun et al.[15] was used. Solvent A was 5% formic acid in distilled water, and solvent B was pure MeOH. The flow rate of the solvent was 1 mL $min^{-1}$, and the wavelength of the UV detector was set to 520 nm. After 20 $\mu$L of sample was injected, the linear gradient of HPLC solvent was used for separating anthocyanins over a period of 30 min as follows. Solvent B was increased from 20 to 40% for 5 min, then from 40 to 60% for 15 min, from 60 to 99% for 5 min, and from 99 to 100% for 5 min, and, finally, returned to the initial conditions. HPLC grade solvent with >99.9% purity and distilled water were used for the mobile phase. Standards of nine anthocyanins (cyanidin-3-glucoside, pelargonidin-3-glucoside, peonidin-3-glucoside, malvidin-3-glucoside, delphinidin, cyanidin, pelargonidin, peonidin, and malvidin) were purchased from Sigma-Aldrich (USA). The anthocyanin standards were dissolved in 0.1% HCl in MeOH at the following concentrations: 1, 50, and 100 $\mu$g $mL^{-1}$. A high linearity ($r^2 > 0.999$) was obtained from each curve (Table 2). Standard compounds were identified on the basis of their retention times, and their concentrations were calculated by comparing the peak areas of samples to those of standards.

**Statistical Analysis.** In this study, statistical analyses were performed using the general linear model (GLM) procedure of the 2005 SAS package (version 9.1; SAS Institute Inc., Cary, NC, USA). The experimental design was completely randomized with duplicates, and the least significant difference test was performed with a 0.05 probability level.

## ■ RESULTS AND DISCUSSION

**Isoflavones in Soybean Seeds of Different Planting Dates.** Isoflavones in soybean seeds of five cultivars (Cheongjakong 3, Somyungkong, Daewonkong, Taekwangkong, and Hwanggeumkong) of three different planting dates

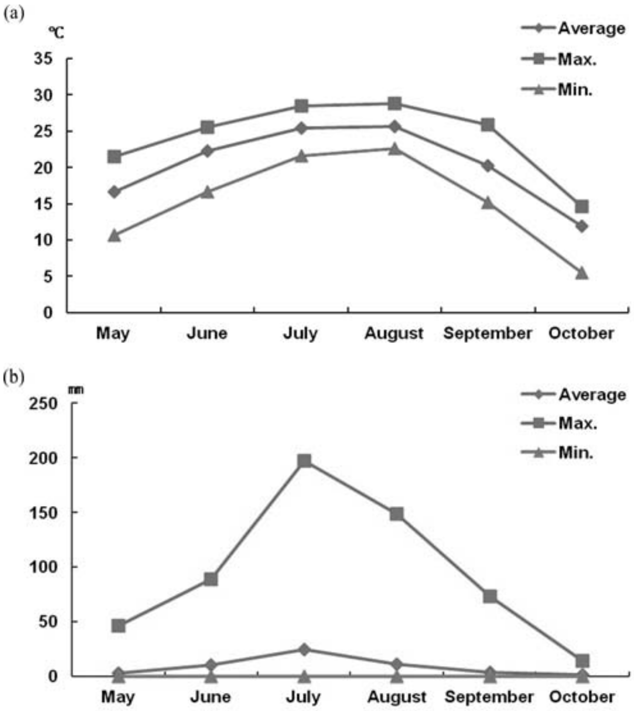


Figure 1. Temperature and precipitation climate conditions of the Yeoju region.

were analyzed, and temperature and precipitation climate conditions of the Yeoju region cultivated soybean varieties are shown in Figure 1 [Agricultural Weather Information Service, Rural Development Administration (RDA), Suwon, Korea]. Among all of the soybean seeds, Somyungkong, planted at normal seed time (June 21), accumulated the highest (7657 $\mu$g g$^{-1}$) concentration of total isoflavones. Somyungkong, planted at late seed time (July 10), also accumulated a higher concentration of total isoflavones (7507 $\mu$g g$^{-1}$) compared to the other soybean seeds (Table 3). In contrast, Cheongjakong 3, planted in late May (May 25), had the lowest concentration of total isoflavones (1519 $\mu$g g$^{-1}$). The effects of planting date on the total isoflavone concentration were monitored. Among the three different planting dates, the highest concentration of average total isoflavones (4098 $\mu$g g$^{-1}$) was observed in early July (July 10), whereas the lowest concentration (3238 $\mu$g g$^{-1}$) was observed in late May (May 25). The average concentration

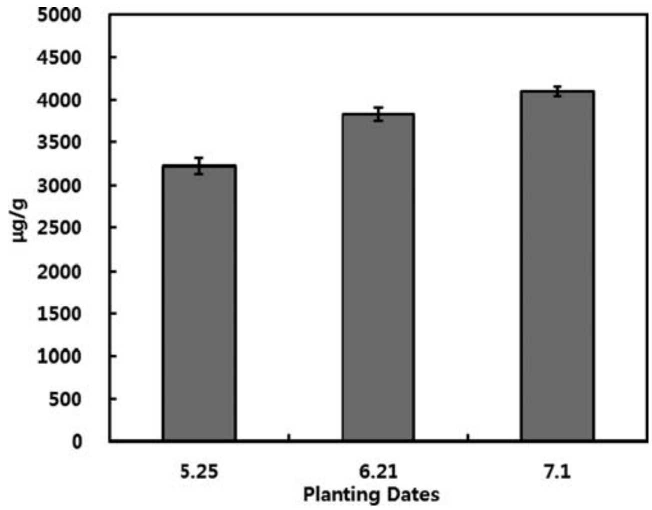


Figure 2. Average total isoflavone concentration in soybean seeds according to planting date.

Table 3. Comparison of Isoflavone Contents (Micrograms per Gram) in Soybean Seeds of Three Different Planting Dates

| | glycoside[a] | | | malonylglycoside[a] | | | acetylglycoside[a] | | | aglycone[a] | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| variety[b] | Din | Gly | Gin | Mdin | Mgly | Mgin | Acdin | Acgly | Acgin | Dein | Glein | Gein | total |
| | | | | | | Planting Date: May 25 | | | | | | | |
| a | 74.0 | 8.4 | 2.2 | 611.7 | 183.9 | 644.4 | 12.1 | 9.4 | 9.6 | 7.5 | 4.0 | 14.4 | 1518.6 |
| b | 460.5 | 87.5 | 62.2 | 2469.2 | 1050.3 | 2320.5 | 49.1 | 61.5 | 24.5 | 27.7 | 4.9 | 26.9 | 6644.8 |
| c | 257.0 | 50.3 | 31.1 | 1666.1 | 443.0 | 1185.3 | 12.7 | 18.4 | 14.6 | 21.5 | 35.1 | 69.0 | 3803.9 |
| d | 90.7 | 20.7 | 10.2 | 556.6 | 242.7 | 665.0 | 13.4 | 13.0 | 9.3 | 4.3 | 3.5 | 5.5 | 1635.0 |
| e | 105.0 | 19.8 | 14.7 | 1023.7 | 224.8 | 975.0 | 15.7 | 37.2 | 22.0 | 16.1 | 9.9 | 60.0 | 2524.0 |
| | | | | | | Planting Date: June 21 | | | | | | | |
| a | 190.4 | 24.4 | 20.4 | 1648.0 | 238.7 | 909.8 | 24.3 | 24.9 | 17.3 | 4.2 | 3.6 | 34.0 | 3140.1 |
| b | 590.7 | 117.2 | 57.0 | 3397.5 | 1000.8 | 2316.1 | 37.2 | 42.4 | 20.4 | 34.6 | 8.5 | 34.5 | 7657.0 |
| c | 100.3 | 30.5 | 24.6 | 616.3 | 260.2 | 684.0 | 37.4 | 27.6 | 18.0 | 19.0 | 11.6 | 32.3 | 1861.8 |
| d | 246.3 | 42.5 | 18.2 | 1625.9 | 290.3 | 823.7 | 20.5 | 21.5 | 12.0 | 47.1 | 6.7 | 72.8 | 3227.5 |
| e | 181.5 | 46.9 | 26.3 | 1494.5 | 318.3 | 1106.1 | 28.1 | 14.5 | 13.0 | 2.2 | 1.7 | 24.9 | 3257.9 |
| | | | | | | Planting Date: July 10 | | | | | | | |
| a | 194.5 | 31.2 | 8.9 | 1401.0 | 300.3 | 892.7 | 24.1 | 18.5 | 10.6 | 1.8 | 1.3 | 15.1 | 2900.0 |
| b | 672.2 | 150.8 | 91.7 | 3979.1 | 733.2 | 1628.3 | 29.6 | 11.2 | 12.0 | 84.3 | 0.8 | 113.5 | 7506.7 |
| c | 227.0 | 46.3 | 22.5 | 1365.0 | 250.3 | 648.1 | 17.3 | 18.3 | 8.6 | 57.8 | 2.6 | 72.9 | 2736.7 |
| d | 234.4 | 40.9 | 31.9 | 1409.8 | 526.7 | 1333.8 | 36.8 | 51.6 | 25.1 | 18.5 | 5.5 | 36.1 | 3751.0 |
| e | 281.2 | 32.3 | 21.3 | 1814.2 | 371.0 | 976.0 | 23.9 | 12.6 | 18.8 | 4.9 | 4.5 | 36.9 | 3597.5 |
| CV (%) | 1.9 | 4.2 | 11.9 | 1.3 | 1.1 | 1.5 | 32.7 | 51.1 | 31.4 | 24.5 | 62.3 | 45.4 | 2.5 |
| LSD $_{(0.05)}$ | 11.2 | 4.7 | 7.9 | 49.1 | 10.9 | 36.9 | 18.6 | 29.3 | 10.9 | 13.1 | 9.8 | 43.9 | 203.7 |

[a]Din, daidzin; Gly, glycitin; Gin, genistin; Mdin, malonyldaidzin; Mgly, malonylglycitin; Mgin, malonylgenistin; Acdin, acetyldaidzin; Acgly, acetylglycitin; Agin, acetylgenistin; Dein, daidzein; Glein, glycitein; Gein, genistein. [b]a, Cheongjakong 3; b, Somyungkong; c, Daewonkong; d, Taekwangkong; e, Hwanggeumkong.

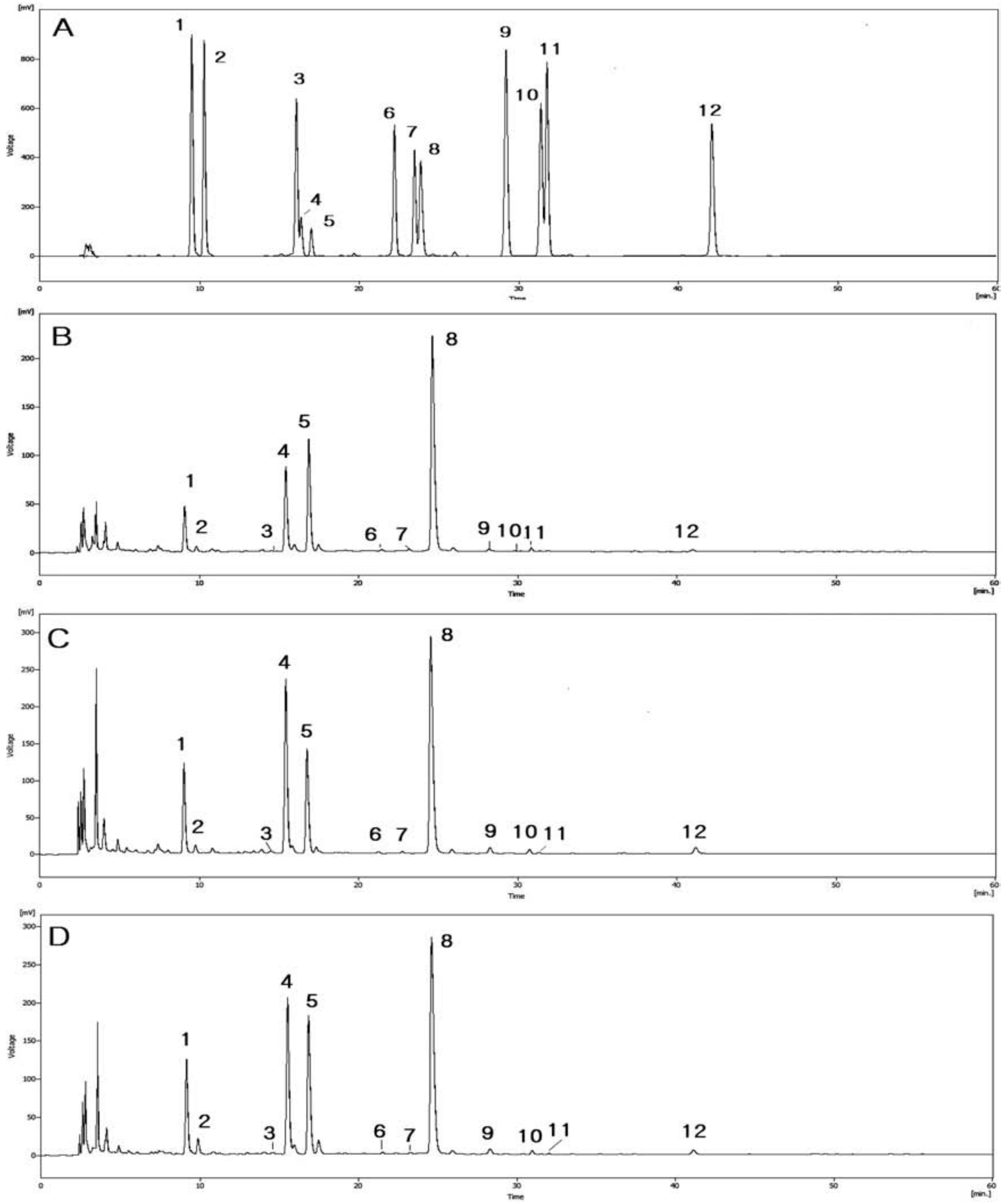


**Figure 3.** Chromatograms showing the results of high-performance liquid chromatography of isoflavones in Choengjakong 3 of different planting dates: (A) isoflavone standards; (B) late May (May 25) planting date; (C) late June (June 21) planting date; (D) early July (July 10) planting date. Peak: 1, daidzin; 2, glycitin; 3, genistin; 4, malonyldaidzin; 5, malonylglycitin; 6, acetyldaidzin; 7, acetylglycitin; 8, malonylgenistin; 9, daidzein; 10, glycitein; 11, acetylgenistin; 12, genistein.

of total isoflavones was therefore approximately 27% higher in early July than in late May (Figures 2, 3). The concentration of average total isoflavones in seeds planted in late May (May 25), late June (June 21), and early July (July 10) varied from 1518 to 6645 $\mu g\ g^{-1}$, from 1862 to 7657 $\mu g\ g^{-1}$, and from 1517 to 6645 $\mu g\ g^{-1}$, respectively. In all of the soybean seeds, malonyldaidzin (1665 $\mu g\ g^{-1}$) and malonylgenistin (1146 $\mu g\ g^{-1}$) were present at higher concentrations, whereas glycitein was detected at the lowest concentration (6.6 $\mu g\ g^{-1}$). The concentration of daidzin was also higher (322 $\mu g\ g^{-1}$) in early July, but significantly lower (197 $\mu g\ g^{-1}$) in late May. On the other hand, glycitein concentrations were lower than the average total isoflavone concentration. The average concentrations of malonyldaidzin in late May, late June, and early July were 1266, 1756.4, and 1994 $\mu g \cdot g^{-1}$, respectively. Malonylglycosides represented 87% of the concentration of total average isoflavones in all of the soybean seeds, whereas acetylglycosides constituted only 1.8%. A previous study showed that isoflavones are primarily present in their storage form as malonylglycosides, whereas acetylglycosides and aglycones are present at low levels.[16] In agreement with this finding, the proportions of malonylglycosides as a percentage of total

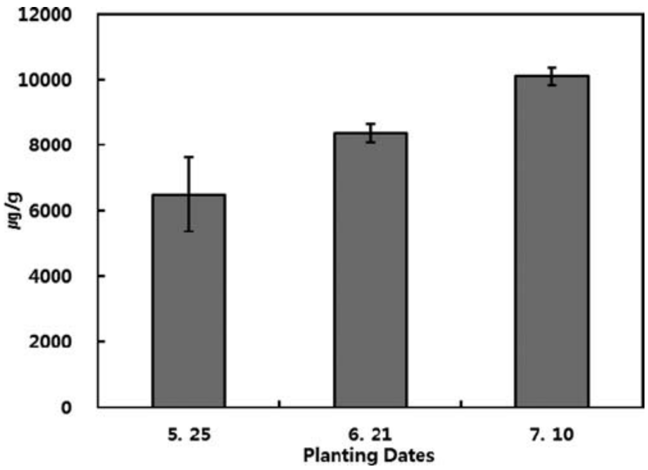


**Figure 4.** Average total anthocyanin concentration in Choengjakong 3 according to planting date.

average isoflavones of soybean seeds in late May, late June, and early July were 88, 87, and 86%, respectively.

Differences in isoflavone concentrations have been observed among planting dates in previous studies.[17] Tsukamoto and coworkers[17] reported that isoflavone concentrations were higher when soybeans were sown in July rather than planted in May. The authors hypothesized that higher isoflavone concentrations in seeds with later planting dates could result from lower temperatures and higher precipitation during pod development and seed-filling periods. Low temperatures and high precipitation during seedpod development are known to favor higher isoflavone contents.[18] In a previous paper, isoflavone concentrations in soybeans are reportedly higher in seeds planted later in the season. The concentration of average total isoflavones of seeds sown in mid June was higher (849 $\mu$g g$^{-1}$) than those sown in late May (1175 $\mu$g g$^{-1}$),[19] whereas another previous paper reported the variation of isoflavone concentrations in soybean seeds showed negative correlation with temperature during the entire growth period.[20] In the present study, the seeds planted in July showed accumulation of the highest concentrations of total isoflavones. On the basis of these results, we conclude that lower temperatures during seedpod development affect the total content and synthesis of isoflavone compounds. In particular, low temperature during the seedpod development stage can lead to high isoflavone content in soybean seeds. Thus, the isoflavone contents of soybean seeds are affected by planting date owing to changes in the environmental conditions. The results of this study can provide useful information to breeders, researchers, and farmers about planting dates and harvest times to obtain high concentrations of phytochemicals including isoflavones in soybean seeds.

**Anthocyanins in Soybean Seeds of Different Planting Dates.** Among the five soybean cultivars, Cheongjakong 3 had a black seed coat color and was the only cultivar in which anthocyanins were detected. Among the three different planting dates, the late planting date (July 10) showed accumulation of the highest concentration of average total anthocyanins (10103 $\mu$g g$^{-1}$), whereas the early planting date (May 25) had the lowest concentration of average total anthocyanins (6489 $\mu$g g$^{-1}$) (Figures 4, 5). The effects of planting date were determined for all anthocyanins. Among the anthocyanin compounds, cyanidin-3-glucoside (6369 $\mu$g g$^{-1}$) content was the highest, followed by malvidin-3-glucoside (564 $\mu$g g$^{-1}$) and peonidin-3-glucoside (603 $\mu$g g$^{-1}$). Delphinidin was present at the lowest concentration of all anthocyanins tested (Table 4). Cyanidin-3-glucoside was present in the highest proportion (76%) of the average total anthocyanins. Furthermore, the contents of cyanidin-3-glucoside and other anthocyanin compounds were influenced by planting dates with different climate conditions. The concentrations of cyanidin-3-glucoside were highest at the late planting date (July 10). These data suggest that anthocyanin concentrations are higher in seeds subjected to low temperatures and relatively higher precipitation levels during seedpod development. Previous studies showed that phytochemicals such as isoflavones and phenolics of soybean seeds vary with temperature and precipitation,[21] and plant phenylpropanoid metabolism can change with environmental conditions.[22] Abiotic and biotic stresses can also alter the phenolic composition of plants.[23] Another previous paper indicated that isoflavone and anthocyanin contents in soybean seeds are affected by soybean genotype, planting dates, and environmental conditions.[24] Thus, on the basis of the results of the present study, we conclude that anthocyanin concentrations vary with environmental factors such as temperature and precipitation during seedpod development. This result indicated that the soybean seed of late planting dates revealed highest concentrations of total anthocyanins due to lower temperatures during seedpod development. The results of the present study would be useful in determining the optimal planting date and harvest time at which anthocyanins reach maximum concentrations. Also, these results should be of interest to researchers and farmers for further investigating the basis of environmental control of soybean seed anthocyanins.

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### Notes

The authors declare no competing financial interest.

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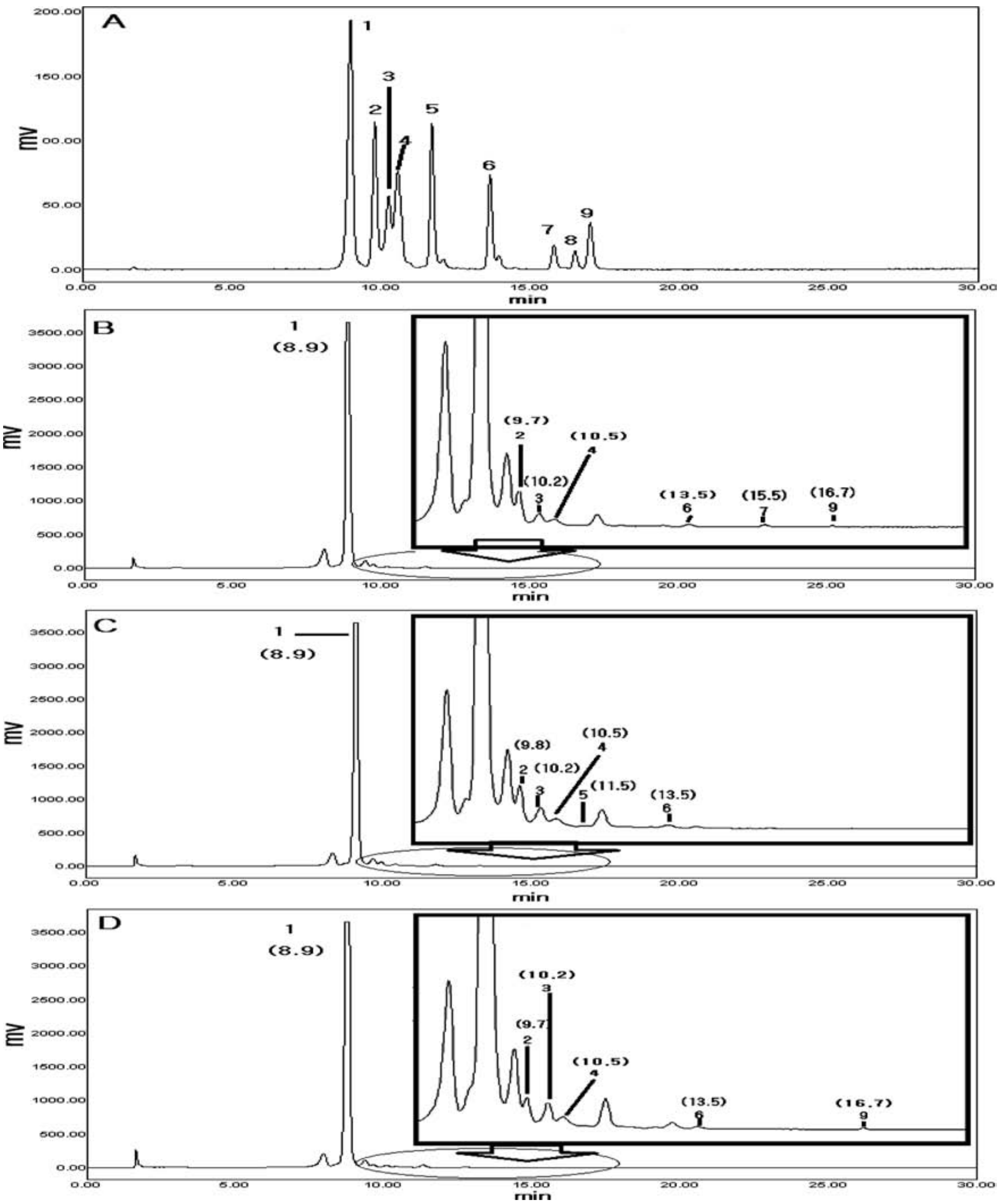


Figure 5. Chromatograms showing the results of high-performance liquid chromatography of anthocyanins in Choengjakong 3 of different planting dates: (A) anthocyanin standards; (B) late May (May 25) planting date; (C) late June (June 21) planting date; (D) early July (July 10) planting date. Peaks: 1, cyanidin-3-glucoside; 2, pelargonidin-3-glucoside; 3, peonidin-3-*O*-glucoside (P3G); 4, malvidin-3-glucoside; 5, delphinidin; 6, cyanidin; 7, pelargonidin; 8, peonidin, 9, malvidin. Numbers in parentheses indicate retention time (RT).

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**Table 4. Comparison of Anthocyanins Contents (Micrograms per Gram) in Choengjakong 3 in Seeds Planted on Different Dates**

| | planting date/100-seed wt[a] | | | | |
|---|---|---|---|---|---|
| anthocyanin | May 25/ 30.9 g | June 21/ 31.4 g | July 10/ 32.8 g | CV (%) | LSD (0.05) |
| cyanidin | 183 | 179 | 180 | 2.6 | 14.7 |
| cyanidin-3-glucoside | 4878 | 6236 | 7993 | 10.0 | 2031.5 |
| delphinidin | nd | 102 | nd | 20.7 | 22.3 |
| malvidin | 155 | nd | 171 | 3.7 | 12.8 |
| malvidin-3-glucoside | 567 | 545 | 580 | 2.4 | 43.9 |
| pelargonidin | 151 | nd | nd | 0.7 | 1.2 |
| pelargonidin-3-glucoside | tr | 626 | 602 | 6.0 | 35.9 |
| peonidin-3-glucoside | 554 | 612 | 642 | 2.8 | 115.2 |
| total | 6489 | 8367 | 10103 | 8.3 | 2189 |

[a]nd, not detected; tr, trace.

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