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# Variation in Isoflavone of Soybean Cultivars with Location and Storage Duration

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Fifteen soybean [*Glycine max* (L.) Merrill] cultivars were grown in Seoul, Suwon, and Kyongsan, Korea, in 1998, 1999, and 2000, and their isoflavone contents were assessed. After harvest, the beans were stored for 3 years at room temperature. Soybean isoflavones were analyzed using high-performance liquid chromatography (HPLC) within each crop year and after storage. Total isoflavone contents ranged from 188.4 to 685.6 mg 100 $g^{-1}$ in 1998, from 218.8 to 948.9 mg 100 $g^{-1}$ in 1999, and from 293.1 to 483.0 mg 100 $g^{-1}$ in 2000. The year × variety, and year × location × variety interactions were significantly different in 1998, the year × location, year × variety, and year × location × variety interactions were significantly different in 1999, and the year × variety interaction was significantly different in 2000 for total and individual isoflavone contents. Total isoflavone contents of soybeans stored for 1 year were only slightly higher than those of soybeans stored for 2 or 3 years. However, the concentrations of individual isoflavones, especially 6″-*O*-malonyldaidzin and 6″-*O*-malonylgenistin, decreased markedly in soybeans stored for 2 or 3 years. These data suggest that it may be feasible to improve soybean cultivars with higher antioxidative substances.

**KEYWORDS: Soybean; variety; isoflavones; HPLC**

## INTRODUCTION

The soybean [*Glycine max* (L.) Merrill], a major upland food crop for the summer season in Korea, is popular because of its good quality protein and oil content, and it has long been part of the Korean diet (*1*). The health benefits of soybean as a food have been well-known for many years and are widely recognized around the world (*2*). Currently, the demand for "health foods" or "functional foods" is increasing in many countries. The soybean provides potential benefits for several of the most common diseases afflicting humans, including an anticancer effect (*1*). Many studies have been conducted on the positive aspects of various soybean components possessing biological characteristics, such as anticarcinogens; antiatherosclerotic, blood glucose lowering, antibacterial (*1*), antioxidative (*3*), and antifungal agents (*4*); tyrosine protein kinase inhibitors (5); and an aromatase-inhibiting (6) cancer preventive property (*1*, *7*). Isoflavones, the important secondary metabolic compounds of soybean, may play essential roles in preventing certain cancers (*1*, *8*, *9*) and reducing the risk of cardiovascular diseases (*10*), improving bone health (*11*), inhibiting the growth of human breast cancer and prostate cancer cell lines in culture (*12*), and possessing antiestrogenic activity (*13*, *14*).

The isoflavone chemical structure is transformed by malonylglycoside and acetylglycoside functional groups. The malonylated isoflavone glycosides are major isoflavone constituents in the soybean seed, are thermally not fixed, and convert into their corresponding isoflavone glycosides (*15*−*17*). Isoflavonoids have structures similar to both physiological and synthetic estrogens and have estrogenic activity in animal (*18*). The efficiency of the anticancer potential for soybean isoflavones was estimated for genistein, which inhibits protein tyrosine kinase and binds weakly to estrogen receptors (*19*). A study into the effectiveness of daidzein found that urinary recovery of daidzein was significantly greater than that of genistein (*20*).

Much attention has been paid to the isoflavone analysis of soybean cultivars and soybean foods (*15*, *16*, *21*−*24*). Twelve isomers were analyzed, 3 aglycons (daidzein, genistein, and glycitein) and 9 glucosides (daidzin, genistin, glycitin; 6″-*O*-acetyldaidzin, -genistin, and -glycitin; and 6″-*O*-malonyldaidzin, -genistin, and -glycitin), in 29 commercial soybean foods (*15*). Wang and Murphy (*16*) reported that the major isoflavone components were daidzin, 6″-*O*-malonyldaidzin, genistin, and 6″-*O*-malonylgenistin in the isoflavone composition of American and Japanese soybean varieties. They also indicated that the total isoflavone contents of a single soybean cultivar ranged

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from 117.6 to 330.9 mg 100 $g^{-1}$ among years and from 117.6 to 174.9 mg 100 $g^{-1}$ among locations within the same year. Hoeck et al. (*21*) studied the role of genotype, environment, and genotype × environment interactions on the isoflavones with six soybean cultivars over 2 years. Tsukamoto et al. (*22*) reported that factors affecting isoflavone contents of soybean seeds grown in a growth chamber included site, planting date, and temperature during seed development. The concentrations of these isoflavones in soybean seeds and cotyledons are thought to reflect the genetic variation of the cultivar containing the compounds (*23*). These researchers mainly analyzed the amounts of isoflavone on the soybean cultivars at the same locations. More recently, Lee et al. (*24*) reported the analysis of the effects on isoflavone variations with different soybean genotypes, crop years, and sites and the relative importance of their interactions by using the GGE biplot program. Their study exhibited environmental main effects (mainly year main effect and year by site interactions) and genotype by environment interaction (mainly genotype by year and genotypes by year by site interactions) were the most important sources of variation for various isoflavones.

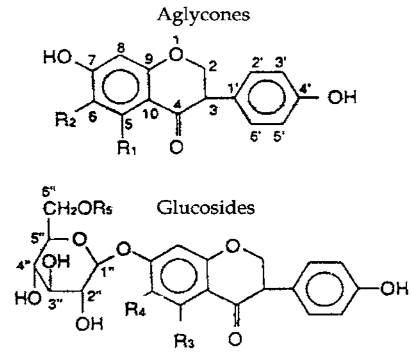
Little information is available on the variation of isoflavones of soybean cultivars with different storage periods, although isoflavones in soybean may possess various biological characteristics. This study was undertaken to evaluate the variation of the isoflavone contents of 15 Korean soybean cultivars by different genotypes, crop years, and sites and the relative importance of their interactions. It also examined changes in isoflavone distributions and contents over different storage periods. The results of this study also supply basic information for breeding soybean cultivars with higher isoflavone contents and for changes in isoflavones during storage.

## MATERIALS AND METHODS

The 15 soybean cultivars used in this experiment were grown at three Korean locations: Seoul, Suwon, and Kyongsan in 1998, 1999, and 2000. These cultivars were selected because they were recommended for growing in Korea. The analysis of isoflavones was carried out at the College of Agriculture and Life Science, Konkuk University, for the 15 cultivars grown in each crop year and afterward stored for 3 years. The harvested samples were stored at room temperature (25 °C), and all other procedures were applied according to the standard methods of soybean cultivation in Korea.

**Analysis of Isoflavones.** *Isoflavone Standards.* Genuine standards of daidzin, daidzein, genistin, and genistein were purchased from a commercial source (Aldrich Chemical Co.). Glycitin, glycitein, 6″-*O*-malonyldaidzin, 6″-*O*-malonylgenistin, and 6″-*O*-malonylglycitin were isolated and purified using the modified method of Wang and Murphy (*15*). The nine reference compounds were chromatographed alone and in mixtures. Retention times of the standard compounds and the major peaks in the extract were recorded.

*Isoflavone Extraction and HPLC Analysis.* The extraction of soybean isoflavones employed the method of Lee et al. (*24*). Two grams of ground soybean seed with the seed coat was mixed with 2 mL of 0.1 N HCl and 10 mL of acetonitrile (ACN) in a 125 mL screw-top flask, stirred for 2 h at room temperature, and filtered through a Whatman no. 42 filter paper. The filtrate was dried under vacuum at a temperature below −30 °C and then redissolved in 10 mL of 80% HPLC grade methanol in distilled water. The redissolved sample was filtered through a 0.45 $\mu$m filter unit (Cameo 13N syringe-filter, nylon) and then transferred to 1 mL vials. The HPLC analysis was conducted using the method of Wang and Murphy (*15*, *16*). A Young-Lin HPLC system equipped with a YMC AM-303 (ODS, 250 × 4.6 mm i.d.) column and a Micromeritics 725 autoinjector with a 20 $\mu$L sample loop was employed. A linear HPLC gradient was used: solvent A was 0.1% glacial acetic acid in distilled water, and solvent B was 0.1% glacial acetic acid in ACN. Following the injection of 20 $\mu$L of the sample, 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. The wavelength of the UV detector was set at 256 nm. HPLC grade solvents and distilled water were used for HPLC and were degassed before use. Solvent ratios were expressed on a volume basis.




| $R_1$ | $R_2$ | $R_3$ | $R_4$ | $R_5$ | Compounds |
|---|---|---|---|---|---|
| H | H | | | | Daidzein |
| OH | H | | | | Genistein |
| H | $OCH_3$ | | | | Glycitein |
| | | H | H | H | Daidzin |
| | | OH | H | H | Genistin |
| | | H | $OCH_3$ | H | Glycitin |
| | | H | H | $COCH_3$ | 6"-*O*-Acetyldaidzin |
| | | OH | H | $COCH_3$ | 6"-*O*-Acetylgenistin |
| | | H | $OCH_3$ | $COCH_3$ | 6"-*O*-Acetylglycitin |
| | | H | H | $COCH_2COOH$ | 6"-*O*-Malonyldaidzin |
| | | OH | H | $COCH_2COOH$ | 6"-*O*-Malonylgenistin |
| | | H | $OCH_3$ | $COCH_2COOH$ | 6"-*O*-Malonylglycitin |

**Figure 1.** Classes and chemical structures of isoflavones in the soybean.

**Statistical Analysis.** The soybean was cultivated using a completely randomized design and replicated three times at three locations. The analysis of the isoflavones by HPLC was repeated four times with three extracts in each cultivar. Analyses of variance were performed using the general linear models procedure (GLM) of the SAS software package (*25*). Differences between the means of samples were analyzed by the least significant difference (LSD) test at a probability level of 0.05.

## RESULTS AND DISCUSSION

**Analysis of Isoflavone Contents with Crop Year.** The individual isoflavone contents of each group of soybeans grown at Seoul, Suwon, and Kyongsan in 1998, 1999, and 2000 are given in **Tables 1−3**. The total isoflavone contents of cropped soybeans at Seoul ranged from 240.9 mg 100 $g^{-1}$ (Shinpaldal 2) to 685.6 mg 100 $g^{-1}$ (Hwaeomput) in 1998, from 320.6 mg 100 $g^{-1}$ (Daweon) to 948.9 mg 100 $g^{-1}$ (Jangyeob) in 1999, and from 293.1 mg 100 $g^{-1}$ (Hwaeomput) to 456.3 mg 100 $g^{-1}$ (Taekwang) in 2000 (**Table 1**). At Suwon, the highest contents were 586.4 mg 100 $g^{-1}$ (Taekwang) in 1998, 724.5 mg 100 $g^{-1}$ (Danbaek) in 1999, and 483.0 mg 100 $g^{-1}$ (Pureun) in 2000 (**Table 2**). At Kyongsan, the total isoflavone contents ranged from 188.4 mg 100 $g^{-1}$ (Myeongjunamul) to 545.0 mg 100 $g^{-1}$ (Suwon 157) in 1998, from 318.9 mg 100 $g^{-1}$ (Daweon)

**Table 1.** Total and Individual Isoflavone Contents of 15 Soybean Cultivars from Seoul in 1998, 1999, and 2000

| | | isoflavone (mg 100 g$^{-1}$) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | glucoside | | | malonylglucoside | | | aglycon | | | |
| cultivar | year | daidzin | glycitin | genistin | daidzin | glycitin | genistin | daidzein | glycitein | genistein | total |
| Taekwang | 1998 | 61.1 | 5.2 | 95.5 | 114.6 | 12.8 | 137.9 | 2.0 | 2.0 | 1.4 | 432.5 |
| | 1999 | 70.3 | 13.0 | 103.3 | 172.4 | 24.4 | 224.9 | 22.3 | 0.7 | 0.7 | 631.8 |
| | 2000 | 25.5 | 10.5 | 24.3 | 189.3 | 26.8 | 167.2 | 4.7 | 5.4 | 2.7 | 456.3 |
| Myeongjunamul | 1998 | 31.4 | 2.4 | 44.7 | 91.5 | 10.1 | 94.9 | 0.7 | 1.0 | 0.4 | 277.1 |
| | 1999 | 71.0 | 12.8 | 120.4 | 174.1 | 27.2 | 233.9 | 4.8 | 0.1 | 0.7 | 645.0 |
| | 2000 | 37.2 | 9.7 | 57.1 | 138.5 | 13.7 | 181.6 | 4.6 | 1.7 | 4.0 | 447.9 |
| Danbaek | 1998 | 39.8 | 3.6 | 48.7 | 152.6 | 18.2 | 140.6 | 1.8 | 0.9 | 0.9 | 407.0 |
| | 1999 | 69.0 | 8.1 | 40.8 | 90.3 | 11.9 | 106.2 | 3.1 | 0.7 | 0.5 | 330.6 |
| | 2000 | 27.9 | 18.1 | 32.5 | 151.7 | 33.2 | 172.1 | 3.0 | 0.4 | 2.0 | 440.9 |
| Daweon | 1998 | 90.2 | 5.2 | 98.3 | 173.9 | 16.1 | 156.9 | 3.0 | 2.0 | 1.4 | 546.8 |
| | 1999 | 30.4 | 6.1 | 40.6 | 135.7 | 2.0 | 99.2 | 5.2 | 0.9 | 0.5 | 320.6 |
| | 2000 | 28.6 | 6.5 | 41.7 | 160.1 | 12.0 | 124.4 | 6.0 | 2.8 | 3.1 | 385.3 |
| Muhan | 1998 | 61.0 | 2.7 | 58.8 | 168.2 | 10.5 | 211.2 | 1.6 | 1.6 | 1.3 | 516.8 |
| | 1999 | 9.5 | 9.3 | 108.7 | 232.3 | 166.5 | 229.6 | 5.0 | 1.4 | 0.7 | 763.0 |
| | 2000 | 38.3 | 9.2 | 49.0 | 155.3 | 10.0 | 165.2 | 7.3 | 2.0 | 4.6 | 440.9 |
| Jangyeob | 1998 | 55.9 | 3.3 | 58.5 | 234.4 | 15.0 | 198.5 | 2.4 | 1.3 | 0.9 | 570.2 |
| | 1999 | 94.7 | 15.6 | 110.7 | 233.4 | 166.4 | 228.7 | 97.7 | 1.3 | 0.7 | 948.9 |
| | 2000 | 34.1 | 13.1 | 51.0 | 148.8 | 19.2 | 179.3 | 4.0 | 1.3 | 2.9 | 453.8 |
| Hwangkeum | 1998 | 85.8 | 9.6 | 87.8 | 208.0 | 20.4 | 165.1 | 2.0 | 1.7 | 0.8 | 581.2 |
| | 1999 | 41.5 | 6.3 | 37.7 | 130.3 | 17.4 | 122.3 | 12.4 | 0.7 | 0.4 | 369.1 |
| | 2000 | 27.1 | 7.2 | 40.5 | 131.8 | 11.6 | 187.4 | 4.2 | 2.4 | 3.9 | 416.1 |
| Hwaeomput | 1998 | 58.3 | 7.7 | 64.1 | 277.2 | 29.3 | 243.8 | 2.8 | 1.3 | 1.2 | 685.6 |
| | 1999 | 38.6 | 42.7 | 182.3 | 130.9 | 18.8 | 163.4 | 10.0 | 0.7 | 0.4 | 587.8 |
| | 2000 | 27.8 | 12.3 | 41.9 | 68.9 | 10.8 | 99.1 | 15.4 | 0.0 | 16.9 | 293.1 |
| Pureun | 1998 | 32.6 | 1.9 | 32.8 | 102.9 | 7.8 | 132.6 | 1.5 | 0.9 | 1.0 | 313.9 |
| | 1999 | 49.5 | 25.8 | 52.7 | 133.8 | 30.8 | 129.2 | 2.9 | 1.2 | 0.0 | 425.9 |
| | 2000 | 38.9 | 20.6 | 32.9 | 167.7 | 32.8 | 149.1 | 6.6 | 3.7 | 3.7 | 455.7 |
| Hannam | 1998 | 40.6 | 3.3 | 64.5 | 100.1 | 11.7 | 137.7 | 3.0 | 0.1 | 1.3 | 362.2 |
| | 1999 | 46.4 | 25.4 | 52.3 | 137.4 | 31.6 | 132.9 | 2.8 | 1.2 | 0.2 | 430.1 |
| | 2000 | 35.8 | 7.6 | 38.9 | 174.4 | 13.2 | 147.6 | 12.3 | 0.9 | 5.4 | 435.9 |
| Geomjeongkong 1 | 1998 | 88.1 | 4.9 | 15.3 | 245.7 | 14.5 | 108.3 | 3.4 | 0.3 | 1.9 | 482.2 |
| | 1999 | 90.4 | 65.3 | 116.6 | 202.8 | 44.2 | 182.6 | 4.6 | 1.3 | 1.5 | 709.4 |
| | 2000 | 35.6 | 11.9 | 31.5 | 155.9 | 19.3 | 122.4 | 11.6 | 26.0 | 3.6 | 417.7 |
| Jinpum 2 | 1998 | 44.7 | 1.8 | 70.9 | 110.8 | 6.6 | 142.3 | 1.9 | 1.4 | 1.0 | 381.4 |
| | 1999 | 94.7 | 84.8 | 114.3 | 202.2 | 45.2 | 179.7 | 4.5 | 1.3 | 0.2 | 726.8 |
| | 2000 | 50.3 | 21.8 | 52.7 | 154.4 | 24.8 | 119.3 | 7.3 | 3.0 | 3.6 | 437.2 |
| Suwon 157 | 1998 | 50.1 | 8.0 | 42.9 | 138.6 | 12.1 | 99.1 | 3.3 | 3.2 | 1.0 | 358.2 |
| | 1999 | 12.2 | 75.1 | 108.3 | 247.2 | 43.9 | 185.4 | 5.6 | 0.1 | 0.2 | 677.9 |
| | 2000 | 40.2 | 5.6 | 65.6 | 106.4 | 5.4 | 123.0 | 7.7 | 1.5 | 5.2 | 360.4 |
| Shinpaldal 2 | 1998 | 25.8 | 17.5 | 35.9 | 73.2 | 5.1 | 80.1 | 1.5 | 1.0 | 0.8 | 240.9 |
| | 1999 | 12.1 | 74.6 | 119.2 | 252.4 | 46.5 | 193.4 | 5.8 | 0.9 | 0.2 | 704.8 |
| | 2000 | 59.7 | 21.3 | 72.4 | 108.0 | 16.0 | 108.8 | 9.4 | 5.6 | 6.5 | 407.7 |
| SS2 | 1998 | 33.5 | 2.2 | 50.4 | 101.0 | 7.5 | 114.7 | 1.9 | 1.0 | 1.1 | 313.3 |
| | 1999 | 26.4 | 30.4 | 177.3 | 376.3 | 122.4 | 157.3 | 5.3 | 0.2 | 0.3 | 896.0 |
| | 2000 | 50.2 | 22.0 | 71.0 | 83.4 | 18.6 | 117.0 | 9.8 | 7.4 | 6.8 | 386.0 |
| LSD (0.05) | 1998 | 1.7 | 0.1 | 9.1 | 13.6 | 5.1 | 2.9 | 0.1 | 0.1 | 0.0 | 17.0 |
| | 1999 | 4.2 | 1.8 | 8.0 | 10.0 | 1.9 | 31.5 | 0.1 | 0.2 | 0.0 | 35.9 |
| | 2000 | 7.5 | 5.7 | 10.6 | 28.4 | 7.8 | 32.9 | 4.5 | 7.7 | 5.2 | 40.6 |

to 811.1 mg 100 g$^{-1}$ (Geomjeongkong 1) in 1999, and from 350.7 mg 100 g$^{-1}$ (Hwaeomput) to 473.1 mg 100 g$^{-1}$ (Jinpum 2) in 2000 (**Table 3**). These results indicate significant differences in total isoflavone contents ($p < 0.05$) between different crop years, locations, and cultivars. In particular, the total isoflavone contents of all three locations in 1999 were much higher than those in the other years, which suggests that isoflavone contents are related to crop years, locations, and varieties (*21*) and indicates that crop year affects isoflavone contents more than does location or cultivar. After weather reports had been examined, it was postulated that low temperatures during the ripening period increased the isoflavone contents in 1999. An earlier study (*23*) found that the total isoflavone contents of soybeans ranged from 116.0 to 309.0 mg 100 g$^{-1}$ within one cultivar and from 46.0 to 195.0 mg 100 g$^{-1}$ within the same cultivar at different locations. Even with soybeans grown in the same area, the isoflavone contents varied from year to year. In another study, the variation in total isoflavones ranged from 117.6 to 330.9 mg 100 g$^{-1}$ in Vinton 81 soybeans from 1989 to 1991 and from 117.6 to 174.9 mg

**Table 2.** Total and Individual Isoflavone Contents of 15 Soybean Cultivars from Suwon in 1998, 1999, and 2000

| | | isoflavone (mg 100 g$^{-1}$) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | glucoside | | | malonylglucoside | | | aglycon | | | |
| cultivar | year | daidzin | glycitin | genistin | daidzin | glycitin | genistin | daidzein | glycitein | genistein | total |
| Taekwang | 1998 | 47.3 | 5.1 | 73.8 | 202.9 | 13.6 | 238.5 | 1.9 | 2.0 | 1.3 | 586.4 |
| | 1999 | 64.2 | 11.2 | 90.1 | 156.8 | 24.3 | 228.1 | 21.7 | 0.7 | 0.7 | 597.6 |
| | 2000 | 25.5 | 8.2 | 27.2 | 176.4 | 22.1 | 190.8 | 1.8 | 0.0 | 1.3 | 453.3 |
| Myeongjunamul | 1998 | 26.2 | 3.1 | 37.8 | 67.9 | 12.2 | 71.0 | 0.8 | 1.1 | 0.5 | 220.5 |
| | 1999 | 29.8 | 7.7 | 38.6 | 87.8 | 24.1 | 98.7 | 5.9 | 0.9 | 0.5 | 294.0 |
| | 2000 | 37.1 | 7.3 | 38.9 | 164.1 | 11.5 | 197.4 | 2.9 | 0.0 | 2.7 | 459.1 |
| Danbaek | 1998 | 42.8 | 3.8 | 46.3 | 120.9 | 19.1 | 105.5 | 1.9 | 0.8 | 0.9 | 341.9 |
| | 1999 | 95.4 | 9.6 | 111.0 | 238.4 | 23.5 | 233.6 | 10.9 | 1.4 | 0.7 | 724.5 |
| | 2000 | 19.9 | 17.9 | 30.4 | 158.9 | 37.2 | 183.4 | 0.7 | 0.0 | 0.7 | 449.0 |
| Daweon | 1998 | 14.2 | 3.5 | 60.7 | 112.1 | 12.3 | 105.2 | 2.7 | 1.8 | 1.4 | 313.9 |
| | 1999 | 35.4 | 3.9 | 174.1 | 44.8 | 26.9 | 117.7 | 8.8 | 0.7 | 0.4 | 412.6 |
| | 2000 | 15.0 | 7.0 | 24.9 | 147.7 | 16.5 | 200.9 | 1.1 | 2.9 | 1.2 | 417.0 |
| Muhan | 1998 | 41.3 | 2.8 | 69.6 | 113.6 | 10.8 | 116.6 | 1.5 | 1.4 | 0.9 | 358.4 |
| | 1999 | 49.4 | 24.9 | 52.5 | 136.2 | 31.0 | 130.4 | 3.1 | 1.2 | 0.2 | 428.9 |
| | 2000 | 26.5 | 9.0 | 37.7 | 151.9 | 15.3 | 195.6 | 2.6 | 0.0 | 2.2 | 440.7 |
| Jangyeob | 1998 | 55.7 | 4.5 | 52.2 | 168.3 | 18.3 | 71.3 | 2.5 | 1.6 | 0.9 | 375.2 |
| | 1999 | 95.3 | 15.3 | 116.3 | 208.2 | 41.9 | 186.5 | 4.8 | 1.3 | 0.2 | 669.7 |
| | 2000 | 31.4 | 13.2 | 40.9 | 144.4 | 23.6 | 186.5 | 3.3 | 0.0 | 2.9 | 446.2 |
| Hwangkeum | 1998 | 65.6 | 4.9 | 72.1 | 193.0 | 21.3 | 157.2 | 2.2 | 1.8 | 0.9 | 519.0 |
| | 1999 | 12.1 | 9.8 | 119.9 | 240.0 | 5.8 | 195.0 | 5.9 | 0.9 | 0.2 | 589.6 |
| | 2000 | 19.0 | 6.7 | 39.8 | 106.1 | 16.7 | 224.9 | 1.7 | 0.0 | 2.4 | 417.3 |
| Hwaeomput | 1998 | 40.1 | 3.0 | 59.0 | 135.0 | 13.0 | 173.5 | 1.6 | 1.2 | 1.0 | 427.3 |
| | 1999 | 24.2 | 18.7 | 159.0 | 288.3 | 51.3 | 154.7 | 11.5 | 3.5 | 0.3 | 711.5 |
| | 2000 | 24.0 | 11.0 | 30.0 | 93.4 | 17.7 | 136.5 | 9.1 | 0.0 | 3.9 | 325.6 |
| Pureun | 1998 | 45.1 | 3.0 | 63.3 | 130.3 | 10.8 | 165.5 | 2.1 | 0.7 | 1.4 | 422.1 |
| | 1999 | 61.3 | 24.3 | 147.9 | 124.2 | 19.1 | 158.5 | 2.4 | 3.5 | 0.2 | 541.3 |
| | 2000 | 40.9 | 20.1 | 36.5 | 185.7 | 28.9 | 164.9 | 3.5 | 1.0 | 1.7 | 483.0 |
| Hannam | 1998 | 36.5 | 3.4 | 80.4 | 84.8 | 11.6 | 103.1 | 2.1 | 0.2 | 1.0 | 323.1 |
| | 1999 | 78.8 | 14.2 | 97.8 | 220.0 | 29.6 | 238.4 | 11.6 | 1.4 | 0.7 | 692.5 |
| | 2000 | 31.6 | 6.0 | 23.6 | 207.5 | 22.2 | 165.2 | 4.9 | 0.9 | 1.8 | 463.6 |
| Geomjeongkong 1 | 1998 | 57.3 | 5.0 | 73.2 | 160.2 | 16.7 | 152.6 | 3.4 | 0.5 | 1.7 | 470.4 |
| | 1999 | 78.2 | 14.2 | 105.9 | 219.2 | 29.6 | 262.5 | 11.6 | 1.4 | 0.7 | 723.3 |
| | 2000 | 23.5 | 9.0 | 23.1 | 152.1 | 23.8 | 189.0 | 2.9 | 16.3 | 1.2 | 440.8 |
| Jinpum 2 | 1998 | 46.4 | 1.7 | 67.2 | 124.4 | 6.4 | 138.2 | 2.0 | 1.5 | 1.0 | 389.0 |
| | 1999 | 37.5 | 3.6 | 52.6 | 90.8 | 8.8 | 111.9 | 3.7 | 0.8 | 0.4 | 310.0 |
| | 2000 | 30.8 | 15.5 | 33.4 | 170.1 | 30.9 | 172.7 | 3.9 | 1.0 | 1.5 | 459.9 |
| Suwon 157 | 1998 | 63.6 | 3.1 | 61.7 | 167.1 | 12.7 | 136.4 | 3.44 | 2.4 | 1.3 | 451.5 |
| | 1999 | 84.3 | 15.9 | 82.9 | 216.2 | 56.9 | 169.4 | 4.0 | 2.2 | 0.2 | 692.0 |
| | 2000 | 23.0 | 3.9 | 32.9 | 124.2 | 8.3 | 185.5 | 1.8 | 0.0 | 1.6 | 381.2 |
| Shinpaldal 2 | 1998 | 23.0 | 16.3 | 35.3 | 73.2 | 5.0 | 76.5 | 1.5 | 0.9 | 0.8 | 232.4 |
| | 1999 | 23.7 | 4.2 | 33.2 | 68.1 | 8.0 | 78.5 | 1.5 | 1.5 | 0.2 | 218.8 |
| | 2000 | 37.6 | 18.0 | 47.5 | 163.2 | 32.7 | 164.0 | 2.3 | 0.9 | 1.6 | 467.7 |
| SS2 | 1998 | 33.0 | 2.1 | 50.5 | 92.7 | 7.6 | 114.7 | 1.9 | 1.0 | 1.1 | 304.4 |
| | 1999 | 39.9 | 7.0 | 56.1 | 87.0 | 8.7 | 89.4 | 1.7 | 2.4 | 0.2 | 292.4 |
| | 2000 | 35.1 | 24.5 | 42.5 | 136.9 | 35.1 | 150.2 | 3.9 | 1.1 | 2.8 | 432.1 |
| LSD (0.05) | 1998 | 0.6 | 0.1 | 16.6 | 5.2 | 0.4 | 5.6 | 0.1 | 0.0 | 0.0 | 18.6 |
| | 1999 | 0.6 | 0.6 | 8.7 | 3.7 | 0.4 | 15.3 | 0.1 | 0.0 | 0.0 | 18.4 |
| | 2000 | 3.2 | 3.8 | 6.2 | 13.1 | 6.8 | 14.5 | 2.4 | 1.4 | 1.2 | 19.6 |

100 g$^{-1}$ with soybeans grown at different locations in 1991. The effect of crop year seemed to have a much greater influence on the variation in isoflavone content than did the location. Moreover, the isoflavone contents in another seven American soybean varieties grown in the United States ranged from 205.3 to 421.6 mg 100 g$^{-1}$ (*16*). Yang and Chung **(***26***)** reported that the range of isoflavone contents of 60 soybean varieties varied from 162.1 to 252.1 mg 100 g$^{-1}$ in 1997 and from 154.4 to 647.0 mg 100 g$^{-1}$ in 1998. The aforementioned data indicate that the minimum and maximum of total isoflavone contents ranged from 46.0 to 421.6 mg 100 g$^{-1}$. In this study, however, the range was from 188.3 to 948.9 mg 100 g$^{-1}$, indicating that Korean soybeans have much higher isoflavone contents than those from other countries. Hence, it was determined that the diversity of isoflavone contents depends on unknown climatic and environmental factors and genetic variation (*22*). To further examine the effect of climatic and environmental factors on isoflavone contents, it is necessary to record climatic data, such as temperature, light duration, and precipitation. Glucoside and malonylglucoside were the major types of each group of

**Table 3.** Total and Individual Isoflavone Contents of 15 Soybean Cultivars from Kyongsan in 1998, 1999, and 2000

| | | isoflavone (mg 100 g$^{-1}$) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | glucoside | | | malonylglucoside | | | aglycon | | | |
| cultivar | year | daidzin | glycitin | genistin | daidzin | glycitin | genistin | daidzein | glycitein | genistein | total |
| Taekwang | 1998 | 38.6 | 4.9 | 60.2 | 149.9 | 14.4 | 181.5 | 1.8 | 2.0 | 1.2 | 454.6 |
| | 1999 | 67.1 | 12.0 | 96.2 | 164.2 | 24.3 | 226.4 | 22.0 | 0.7 | 0.7 | 613.6 |
| | 2000 | 22.1 | 10.8 | 25.5 | 163.8 | 24.7 | 178.7 | 2.0 | 0.0 | 0.9 | 428.5 |
| Myeongjunamul | 1998 | 22.4 | 4.3 | 32.7 | 54.0 | 15.3 | 56.8 | 1.0 | 1.2 | 0.6 | 188.4 |
| | 1999 | 19.3 | 9.6 | 74.5 | 116.7 | 25.5 | 189.5 | 5.3 | 0.2 | 0.6 | 441.0 |
| | 2000 | 32.9 | 8.0 | 43.1 | 159.7 | 12.3 | 184.4 | 3.0 | 0.0 | 2.0 | 445.5 |
| Danbaek | 1998 | 46.3 | 4.1 | 44.0 | 100.1 | 20.1 | 75.3 | 2.0 | 0.7 | 0.9 | 293.6 |
| | 1999 | 80.1 | 8.8 | 59.7 | 131.0 | 15.8 | 146.0 | 4.8 | 0.9 | 0.6 | 447.6 |
| | 2000 | 20.1 | 19.8 | 28.2 | 138.4 | 39.0 | 171.8 | 1.9 | 0.0 | 1.3 | 420.5 |
| Daweon | 1998 | 33.5 | 2.7 | 43.9 | 82.7 | 10.0 | 79.2 | 2.5 | 1.7 | 1.3 | 257.5 |
| | 1999 | 32.7 | 4.8 | 76.5 | 85.8 | 3.8 | 107.6 | 6.5 | 0.8 | 0.4 | 318.9 |
| | 2000 | 19.7 | 9.7 | 30.3 | 143.0 | 0.0 | 184.1 | 1.1 | 2.4 | 0.6 | 391.0 |
| Muhan | 1998 | 31.2 | 2.9 | 35.9 | 85.8 | 11.2 | 80.5 | 1.4 | 1.2 | 0.6 | 250.8 |
| | 1999 | 15.9 | 13.6 | 70.8 | 171.8 | 52.3 | 166.4 | 3.8 | 1.3 | 0.2 | 495.9 |
| | 2000 | 24.1 | 9.0 | 41.0 | 137.3 | 15.8 | 189.5 | 2.1 | 0.0 | 2.2 | 421.0 |
| Jangyeob | 1998 | 55.4 | 7.0 | 47.1 | 131.3 | 23.5 | 43.4 | 2.6 | 2.1 | 0.8 | 313.3 |
| | 1999 | 95.0 | 5.1 | 113.4 | 220.1 | 67.0 | 205.4 | 9.1 | 1.3 | 0.3 | 716.6 |
| | 2000 | 26.5 | 11.4 | 43.7 | 134.9 | 21.4 | 193.7 | 2.3 | 0.0 | 2.7 | 436.5 |
| Hwangkeum | 1998 | 53.1 | 3.3 | 61.2 | 180.0 | 16.5 | 150.1 | 2.4 | 1.8 | 1.1 | 469.6 |
| | 1999 | 61.7 | 11.9 | 57.4 | 253.2 | 8.7 | 150.3 | 8.0 | 0.8 | 0.2 | 552.3 |
| | 2000 | 19.7 | 9.6 | 41.6 | 135.4 | 20.3 | 196.5 | 1.7 | 0.0 | 0.9 | 415.6 |
| Hwaeomput | 1998 | 30.5 | 1.9 | 54.6 | 89.2 | 7.7 | 134.6 | 1.1 | 1.2 | 0.9 | 321.7 |
| | 1999 | 66.6 | 7.0 | 65.3 | 214.1 | 32.4 | 244.6 | 10.7 | 1.2 | 0.4 | 642.3 |
| | 2000 | 26.6 | 17.9 | 26.3 | 108.3 | 25.6 | 139.3 | 5.4 | 0.0 | 1.3 | 350.7 |
| Pureun | 1998 | 73.2 | 6.2 | 11.5 | 177.5 | 18.1 | 220.2 | 3.3 | 0.6 | 2.3 | 512.9 |
| | 1999 | 54.8 | 5.0 | 100.2 | 128.8 | 23.5 | 142.3 | 2.7 | 1.7 | 0.0 | 459.1 |
| | 2000 | 36.8 | 17.8 | 36.8 | 185.9 | 28.4 | 157.6 | 5.2 | 0.0 | 1.4 | 469.8 |
| Hannam | 1998 | 33.2 | 3.6 | 42.9 | 73.6 | 11.5 | 82.5 | 1.6 | 2.0 | 0.9 | 251.6 |
| | 1999 | 58.0 | 18.2 | 67.7 | 169.1 | 30.6 | 170.6 | 4.5 | 1.3 | 0.3 | 520.3 |
| | 2000 | 29.0 | 8.8 | 33.4 | 184.9 | 20.0 | 168.7 | 4.4 | 0.0 | 2.1 | 451.3 |
| Geomjeongkong 1 | 1998 | 42.4 | 5.1 | 48.3 | 115.0 | 19.7 | 101.4 | 3.4 | 2.1 | 1.5 | 338.7 |
| | 1999 | 83.8 | 23.4 | 111.0 | 210.7 | 35.4 | 338.0 | 6.6 | 1.3 | 0.9 | 811.1 |
| | 2000 | 14.4 | 8.3 | 19.3 | 152.9 | 29.7 | 154.8 | 6.8 | 0.0 | 1.3 | 387.5 |
| Jinpum 2 | 1998 | 48.4 | 1.6 | 63.9 | 141.8 | 6.3 | 134.4 | 2.3 | 1.7 | 1.0 | 401.3 |
| | 1999 | 53.7 | 6.8 | 72.0 | 125.3 | 14.7 | 137.9 | 4.1 | 1.0 | 0.2 | 415.8 |
| | 2000 | 25.1 | 15.8 | 30.1 | 186.2 | 31.8 | 178.2 | 2.8 | 1.3 | 1.8 | 473.1 |
| Suwon 157 | 1998 | 87.2 | 1.9 | 56.4 | 166.2 | 7.5 | 218.7 | 3.5 | 1.9 | 1.7 | 545.0 |
| | 1999 | 32.2 | 7.0 | 93.3 | 230.7 | 49.6 | 176.6 | 4.7 | 0.2 | 0.2 | 594.3 |
| | 2000 | 22.0 | 6.3 | 36.8 | 124.8 | 14.9 | 178.1 | 0.6 | 0.0 | 0.8 | 384.2 |
| Shinpaldal 2 | 1998 | 20.7 | 15.2 | 34.7 | 73.2 | 5.0 | 73.2 | 1.4 | 0.9 | 0.7 | 225.0 |
| | 1999 | 39.7 | 7.9 | 51.9 | 107.2 | 13.6 | 111.7 | 2.4 | 1.1 | 0.2 | 335.5 |
| | 2000 | 31.3 | 17.6 | 38.4 | 169.7 | 32.7 | 162.6 | 1.9 | 0.0 | 1.3 | 455.6 |
| SS2 | 1998 | 32.4 | 2.1 | 50.5 | 85.7 | 7.7 | 114.7 | 1.8 | 1.0 | 1.1 | 296.9 |
| | 1999 | 69.4 | 13.7 | 104.7 | 151.2 | 16.3 | 169.2 | 2.6 | 0.4 | 0.2 | 527.5 |
| | 2000 | 28.6 | 18.4 | 36.2 | 162.1 | 34.6 | 156.2 | 1.4 | 0.0 | 0.4 | 437.9 |
| LSD (0.05) | 1998 | 0.8 | 0.1 | 2.4 | 19.6 | 0.4 | 5.8 | 0.1 | 0.1 | 0.0 | 17.8 |
| | 1999 | 17.5 | 2.6 | 3.7 | 1.6 | 0.4 | 33.2 | 0.1 | 0.2 | 0.0 | 38.0 |
| | 2000 | 4.6 | 3.4 | 6.1 | 15.0 | 6.1 | 19.1 | 2.9 | 0.7 | 1.1 | 30.5 |

isoflavone; the forms of aglycon were found only in low amounts when the extraction was examined at room temperature. Heating during the extraction process was a limiting factor for malonylglucoside. **Figure 1** shows the 12 classes of soybean isoflavones, which consist of four groups: glucosides, malonylglucosides, acetylglucosides, and aglycons. Kudou et al. (*17*) and Farmakalidis and Murphy (*27*) reported that malonylglucosides are thermally unstable and convert to acetylglucoside and aglycon. Similar patterns of distribution of isoflavone components among the 15 soybean cultivars were observed in this study. Previous analyses of soybean isoflavones (*15*, *16*, *21*, *26***)** detected daidzin, 6″-*O*-malonyldaidzin, glycitin, 6″-*O*-malonylglycitin, genistin, and 6″-*O*-malonylgenistin in large amounts. However, aglycon was only found in small amounts when the examination was conducted at room temperature (*22*). Moreover, a comparison showed that the amounts of the glycitin group were lower than for the daidzin and genistin groups. The results of this study are very similar to that of a previous study, which included the detection of isoflavone components (*15*, *16*, *26*).

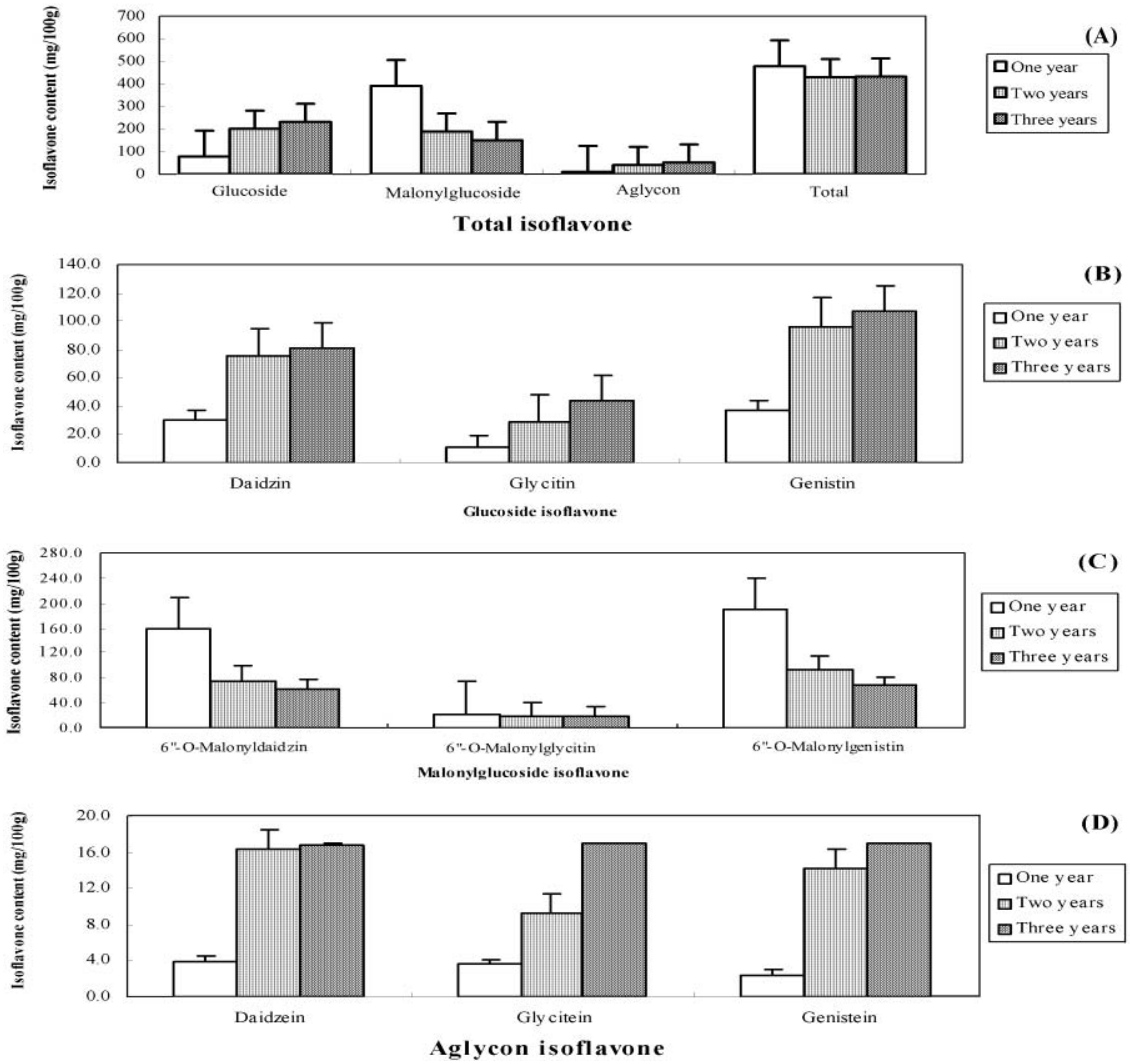



**Figure 2.** Changes in isoflavone composition and contents of soybean cultivars during storage at room temperature.

**Table 4.** Interactions between Locations and Cultivars for Total and Individual Isoflavone Contents of 15 Soybean Cultivars across Three Locations during 1998, 1999, and 2000[a]

| | | 1998 | | | 1999 | | | 2000 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| isoflavone | Y | L | V | L × V | L | V | L × V | L | V | L × V | Y × L × V |
| daidzin | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** |
| glycitin | ** | ** | ** | ** | ** | ** | ** | NS | ** | * | ** |
| genistin | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** |
| 6′′-*O*-malonyldaidzin | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** |
| 6′′-*O*-malonylglycitin | ** | NS | ** | ** | ** | ** | ** | ** | ** | ** | ** |
| 6′′-*O*-malonylgenistin | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** |
| daidzein | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** |
| glycitein | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** |
| genistein | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** |
| total | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** |

[a] Y, year; L, location; C, cultivar; NS, not significant at the 0.05 probability level; * and **, significant at the 0.05 and 0.01 probability levels, respectively.

The interactions of total and individual isoflavone contents of the 15 soybean varieties within each crop year at the three different locations are shown in **Table 4**. Significant interactions of year × location in 1999 were higher than in 1998 or 2000. The results reflect the effect of variable climatic factors on the isoflavone contents of soybean. There were significant differences between locations in the same year for total and individual isoflavone contents. In particular, soybean cultivars had very high concentrations of total isoflavones when grown in Seoul. In both 1998 and 2000, the year × cultivar and year × location × cultivar interactions were significantly different for total and individual isoflavone contents. In 1999, the year × location, year × cultivar, and year × location × cultivar interactions were significantly different for total and individual isoflavone con-

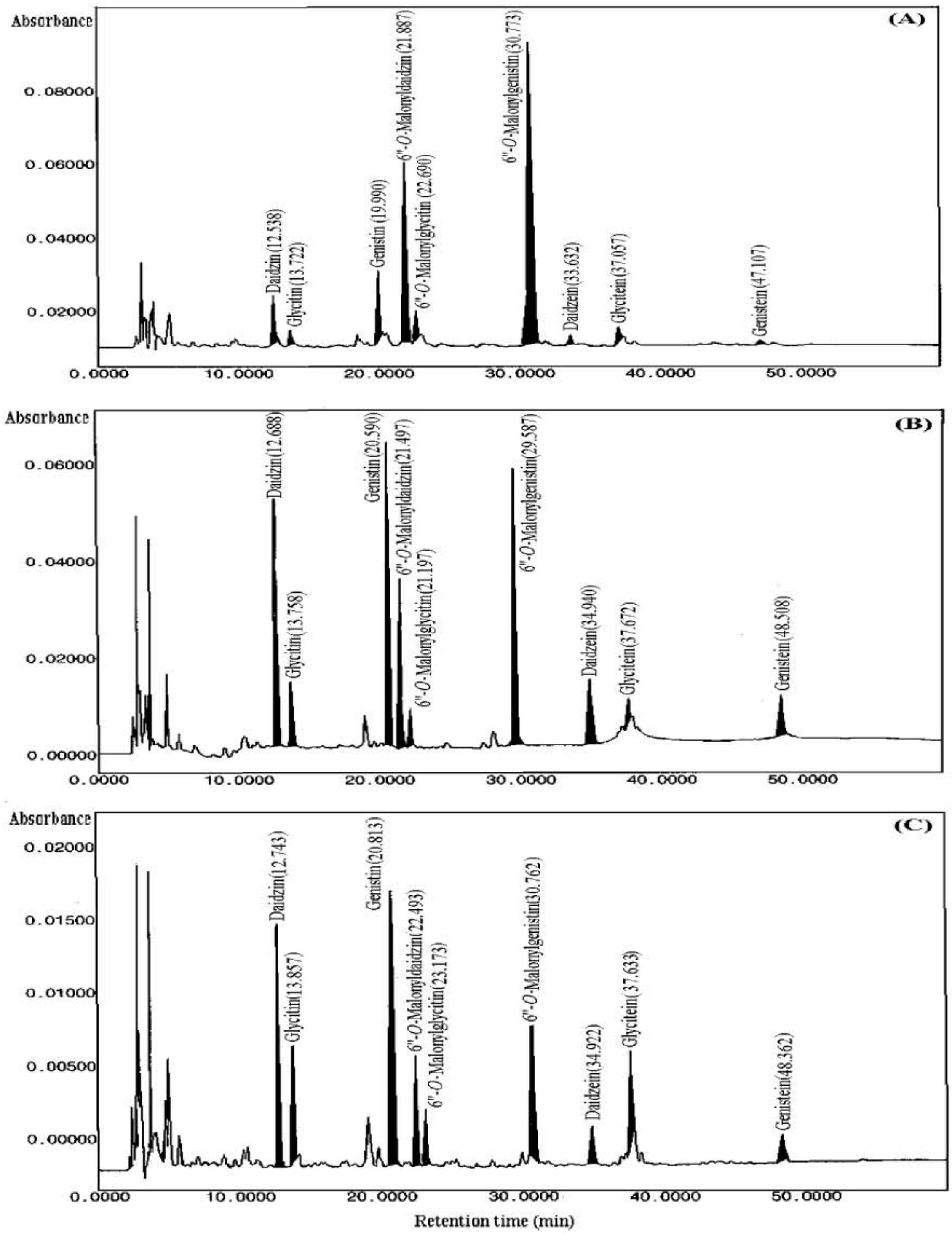



**Figure 3.** Comparison of HPLC chromatograms among 1 year of storage (A), 2 years of storage (B), and 3 years of storage (C) of Geomjeongkong 1 cultivar extracts.

tents. The results of this study are similar to those of Hoeck et al. (*21*) and Lee et al. (*24*), who reported soybean isoflavone contents and interactions between the genotype, genotype × year, genotype × location, and genotype × year × location. Climatic conditions, particularly temperature, might have been the main factor responsible for the variation in isoflavone contents during the seed-ripening phase. It is thought that low temperatures during this period increased the isoflavone contents of the soybean seed. To investigate the influence of temperature on the isoflavone content of soybeans during the growing period, Tsukamoto et al. (*22*) grew soybeans in a temperature-controlled growth chamber. They reported that the isoflavone contents of soybean seed developed at low temperature were considerably higher than those of soybean seed developed at high temperature. The isoflavone contents were significantly higher in 1999 than in 1998 or 2000, possibly because the mean temperature of the seed-ripening period in 1999 was ∼1 °C lower than that of the seed-ripening period in 1998 or 2000.

**Variation of Isoflavone Concentrations with Storage Time in Soybean Seed.** This study showed that the isoflavone concentrations varied with storage years. Total isoflavone concentrations were higher in seed stored for 1 year (477.2 mg

100 g$^{-1}$) than in those stored for 2 (429.3 mg 100 g$^{-1}$) or 3 years (425.4 mg 100 g$^{-1}$). The effect of storage period on isoflavone concentrations was statistically significant for 1, 2, and 3 years. Generally, isoflavones comprise four groups: aglycon, glucoside, malonyl, and acetylglucoside. In this study, aglycon had the lowest concentration, and malonylglucoside had the highest (**Figure 2C**,**D**). The concentrations of glucoside and aglycon increased with storage duration, whereas malonylglucoside decreased after storage for 3 years. The concentration of malonylglucoside decreased by ∼2 times, whereas glucoside and aglycon increased by 3−4 times during storage for 2 years (**Figure 2**). The glucoside group consisted of daidzin and genistin, and these isoflavones increased in concentration during storage for 2 years. The concentrations of the aglycon group showed a variation pattern similar to that of the glucoside group but were about 4 times lower on average (**Figure 2B**,**D**). Among the malonylglucoside group, both 6′′-*O*-malonyldaidzin (167.3 mg 100 g$^{-1}$) and 6′′-*O*-malonylgenistin (199.8 mg 100 g$^{-1}$) had high concentrations after storage for 1 year but decreased over the next 2 years (**Figure 2C**). Generally, the concentrations of nine individual isoflavones either significantly decreased or increased during storage for longer periods. The HPLC chromatograms of Geomjeongkong 1 soybeans stored for 1, 2, and 3 years are shown in **Figure 3**. In this study, it is possible that the high temperatures of the summer season during storage and oxidative reactions influenced isoflavone contents. For this reason, we assumed that the isoflavones of the malonylated type were easily transformed to the glycoside and aglycon groups and that their amounts increased with longer storage durations. Therefore, we need to study the effects of storage environments, such as humidity and temperature, on the transformation of isoflavone groups.

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