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Solvent Effects on Extraction and HPLC Analysis of Soybean Isoflavones and Variations of Isoflavone Compositions As Affected by Crop Season

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Spring (February to June) and fall (August to December) crops of soybean grown yearly in Taiwan with reverse temperature patterns provide a novel model to assess the effect of the crop season. In this study, three soybean cultivars, namely CH 1, VS-KS 2, and HBS, were grown for 2001 fall, 2002 spring, 2003 fall, 2004 spring, 2004 fall, and 2005 spring crops. The harvested and sun-dried soybeans were lyophilized, pulverized, and stored at –25 °C until HPLC analyses of isoflavone compositions were performed. As affected by extraction solvent and HPLC mobile phase, the amount of isoflavones extracted by methanol–H_2O was higher than those extracted by acetic acid–acetonitrile. In addition, when both extracts were subjected to HPLC analysis with reversed C18 column run respectively with methanol–H_2O and acetic acid–acetonitrile mobile phases, malonyldaidzin, malonylglycitin, and malonylgenistin were not detected in the former phase. Accordingly, all harvested soybeans were subjected to methanol–H_2O extraction and HPLC analysis with the acetic acid–acetonitrile mobile phase. Among the detected soybeans, daidzin, genistin, malonyldaidzin, and malonylgenistin were the majors and glycitin, malonylglycitin, daidzein, and genistein were the minors of isoflavones. As affected by crop season for each cultivar grown for 3 years, daidzin, genistin, malonyldaidzin, and malonylgenistin contents of soybeans of the fall crops were significantly higher than those of their spring crops ($p < 0.05$).

KEYWORDS: Soybean; isoflavone; malonyl isoflavones; crop season; HPLC

INTRODUCTION

Soybean is rich in dietary protein, essential fatty acids, and isoflavone contents. Dietary or supplemented isoflavones as phytoestrogens to confer chemopreventive effectiveness of cardiovascular diseases, osteoporosis, menopausal syndrome, and sex-hormone-related cancers (breast, ovary, and prostate) have been demonstrated (*1*–*4*). In global markets, soybean is one of the most affordable sources of protein-rich foods. Since soy isoflavone contents vary considerably depending on variety, location, climate, cultivation practice, and storage (*5*–*8*), selection of productive soybean cultivars with potent isoflavone biosynthesis is important.

In Taiwan, spring and fall crops of soybean are grown annually. Generally, spring crops are planted in February and harvested in June and fall crops are planted in August and harvested in December. The temperature pattern during the planting period of spring and fall crops varies in a reverse manner. For most crops, it is inevitable that their agronomic properties and product characteristics vary more or less between spring and fall crops. In the past, most attention has been focused on yield improvement and cost reduction for soybean production; the variation of soybean isoflavone contents between spring and fall crops has been meagerly investigated. From the viewpoint of development of nutraceutical foods, investigation of isoflavone compositions as affected by crop season is needed. In this study, with an attempt to harvest soybeans from spring and fall crops, three soybean cultivars (non-GMO) were grown for 3 years, namely 2001 fall, 2002 spring, 2003 fall, 2004 spring, 2004 fall, and 2005 spring crops. The harvested and sun-dried soybeans were subjected to isoflavone compositional analyses. For validation of isoflavone extraction and HPLC analysis, samples were respectively subjected to extraction and HPLC analyses with a methanol–H_2O system (*9*) and an acetic acid–acetonitrile system (*10, 11*). On the basis of the validated procedure, all soybeans were subjected to extraction with methanol-H_2O and followed by HPLC analyses with acetic acid-acetonitrile mobile phase for quantitative comparisons.

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MATERIALS AND METHODS

Soybean Cultivation and Preparation. Three soybean cultivars of CH 1 (regular soybean), VS-KS 2 (vegetable soybean), and HBS (black soybean) were cultivated in an experimental field located in Shuishang, Chiayi, Taiwan. Two crops were planted in one-half area and rotated with peanut, sweet potato, or maize each year. Spring crops were planted in February and harvested in June, and fall crops were planted in August and harvested in December. For each crop, a randomized complete block design was applied for the triplicate experiments. Each block (0.6 m × 5 m) was seeded and planted with two lines of soybean (40 cm × 10 cm) and cultivated with regular practices until fully mature for harvest.

After harvest, 10 plants were sampled randomly from each block and dried under sunshine, and the sound seeds were collected. Batches of the harvested soybeans (ca. 20 g) were further lyophilized (Lyvotac GT2, Finn-Aqua, Heraus, Germany), pulverized by a cyclone mill (Krups type 203, Tokyo, Japan), sealed in high-density polyethylene plastic (PE) bags and stored at −25 °C until compositional analyses.

Weather Recording during Soybean Cultivation. During the crop seasons, i.e., from February to June for spring crops and August to December for the fall crops, monthly temperatures were obtained from the Southern Region Weather Center, Central Weather Bureau, Taiwan. The collected and recorded temperatures included the crop seasons of 2001 fall, 2002 spring, 2003 fall, 2004 spring, 2004 fall, and 2005 spring crops.

Validation of Isoflavone Extraction and HPLC Analysis. For comparison and validation, triplicate soybean flour samples from each of three cultivars were respectively subjected to isoflavone extraction by the methanol–H_2O method (*9*) and the acetic acid–acetonitrile method (*10, 11*). The respective extracts were further subjected to HPLC analyses by methanol–H_2O and acetic acid–acetonitrile mobile phases. Details of extraction and HPLC analyses are described as follows.

For the methanol–H_2O method, through using a 10-mL teflon centrifuge tube (Nalgene 2313, Nalge Co., Rochester, NY), 100 mg of each soybean flour was deposited and mixed with 4 mL of 80% methanol. After homogenization with a polytron (PT3000, Kinematica AG, Littau, Switzerland) equipped with an aggregate probe (PT-DA 3007/2) operated at 15 000 rpm for 1 min, an additional 1 mL of 80% methanol was applied for cleaning the probe and pooled. The tubes were screw-capped and heated in a water bath at 70 °C for 30 min. During heating, the tubes were inverted by hand for agitation every 5 min. Then, the tubes were centrifuged (Sigma Labrozentrifugen 2K15, Osterode, Germany) at 20 °C at 8500*g* for 30 min. A 1 mL portion was withdrawn and membrane-filtered (0.45 μm) for analysis with an HPLC system equipped with Hitachi L-7100 pump, Hitachi L-7420 UV–vis detector, and Hitachi L-7445 diode array detector. A reverse phase C18 column (250 × 4.6 mm, 5 μm, Thermal Hypersil ODS, Thermal Hypersil GmbH, Kleinostheim, Germany) was run with a gradient solvent system initiated with 30% of A solvent (methanol) and 70% B solvent (H_2O) to 70% A and 30% B in 16 min and held for an additional 2 min and returned back to the initial 30% A and 70% B in 1 min. The flow rate and injection volume were 1.0 mL/min and 20 μL, respectively.

For the acetic acid–acetonitrile method (*10, 11*), 100 mg of each soybean flour was deposited into a 10-mL teflon centrifuge tube (Nalgene 2313) and mixed with 5 mL of acetonitrile and 1 mL 0.1 N HCl. The tubes were screw-capped and continuously shaken for 2 h. After centrifuging (8500*g*, 15 min at 20 °C), the supernatant was vacuum dried at 30 °C by a rotary evaporator (Eyela N-1000, Tokyo Rikakikai Co., Tokyo, Japan). The extract was dissolved in 5 mL of 80% methanol, membrane filtered (0.45 μm), and subjected to analysis with the HPLC system described above. Each analysis with a reverse C18 column was run with a gradient solvent system initiated with 90% of solvent A (0.1% glacial acetic acid in H_2O) and 10% solvent B (0.1% glacial acetic acid in acetonitrile) to 70% A and 30% B in 60 min, further decreased to 10% A and 90% B in 3 min, and then returned back to the initial 90% A and 10% B in 2 min. The flow rate and injection volume were 1.0 mL/min and 20 μL, respectively. Standard daidzin, glycitin, genistin, daidzein, genistein, and malonylgenistin (Sigma-Aldrich Co., St. Louis, MO) were run under identical conditions for quantitative and qualitative analysis. The other isoflavones, i.e., malonyldaidzin and malonylglycitin were identified according to their retention times and diode array spectra (*10, 11*). The peak area of daidzin was applied as an internal standard for relative quantity estimation of malonyldaidzin and malonylglycitin.

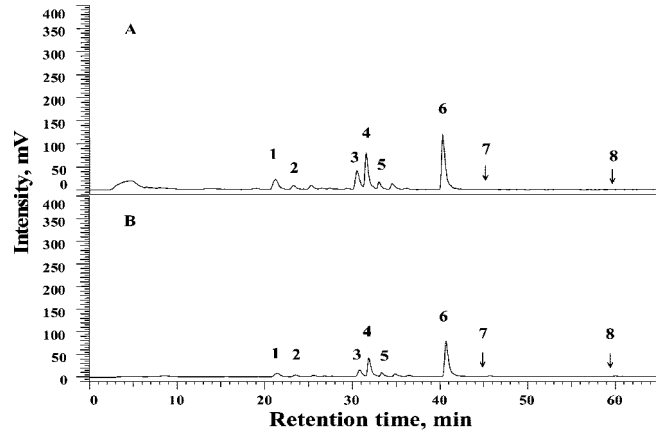

Figure 1. HPLC chromatograms analyzed with the acetic acid–acetonitrile mobile phase of soybean isoflavones extracted with methanol–H_2O (A) and soybean isoflavones extracted with acetic acid–acetonitrile (B); (1) daidzin; (2) glycitin; (3) genistin; (4) malonyldaidzin; (5) malonylglycitin; (6) malonylgenistin; (7) daidzein; (8) genistein.

Determination of Soybean Isoflavone Composition. On the basis of the achieved observation indicating that soybean isoflavones extracted by the solvent methanol–H_2O was slightly higher than those extracted by acetic acid–acetonitrile and the malonyl isoflavones were not detected in HPLC analysis run with methanol–H_2O mobile phase, all soybean samples were subjected to methanol–H_2O extraction and HPLC analysis with the acetic acid–acetonitrile mobile phase. Isoflavone contents were expressed as milligram per gram of freeze-dried soybean flour.

Statistics. In this experiment, three cultivars of soybean have been grown for 3 years including three spring crops and three fall crops. Triplicate experiments for each cultivar in each crop were conducted. Means of determinations with standard deviation are expressed. A statistical analysis system (SAS) was applied for ANOVA analyses.

RESULTS AND DISCUSSION

Spring and fall crops of soybean in Taiwan are generally planted from February to June and from August to December, respectively. Monthly temperatures (data not shown) increased from low to high for the spring crops and decreased from high to low for the fall crops. In particular, during the harvest season of June and December, the temperature difference may exceed 10 °C. Among different years, the monthly temperature difference varied in a limited range.

When soybean samples were respectively subjected to methanol–H_2O and acetic acid–acetonitrile extraction and both extracts were further subjected to HPLC analysis with the methanol–H_2O mobile phase (*9*), daidzin, glycitin, genistin, daidzein, and genistein were detected. However, when the same extracts were subjected to HPLC analysis with the acetic acid–acetonitrile mobile phase (*10, 11*), eight isoflavones including malonyldaidzin, malonylglycitin, and malonylgenistin were detected (**Figure 1**). In comparison, except daidzein and genistein, contents of the other detected isoflavones extracted by methanol–H_2O (**Figure 1A**) were higher than those extracted by acetic acid–acetonitrile (**Figure 1B**). Accordingly, all harvested soybean flour samples were subjected to methanol–H_2O extraction and HPLC analysis with the acetic acid–acetonitrile mobile phase.

Yearly comparisons of the average and standard deviation of daidzin, glycitin, genistin, malonylglycitin, malonyldaidzin,

Table 1. Isoflavone Compositions of Soybeans of CH 1 Cultivar Harvested from 2001 Fall, 2002 Spring, 2003 Fall, 2004 Spring, 2004 Fall, and 2005 Spring Crops

	isoflavone content (mg/g, freeze-dried weight)[a]					
composition	fall 2001	spring 2002	fall 2003	spring 2004	fall 2004	spring 2005
daidzin	0.860 ± 0.023[a]	0.560 ± 0.049[bc]	0.520 ± 0.052[c]	0.353 ± 0.006[d]	0.609 ± 0.035[b]	0.203 ± 0.004[e]
glycitin	0.079 ± 0.004[e]	0.178 ± 0.019[a]	0.129 ± 0.007[c]	0.150 ± 0.005[b][a]	0.136 ± 0.006[bc]	0.102 ± 0.005[d]
genistin	1.223 ± 0.028[a]	0.639 ± 0.052[b]	0.501 ± 0.078[c]	0.436 ± 0.018[d]	0.663 ± 0.039[b]	0.237 ± 0.007[e]
malonyldaidzin	1.503 ± 0.041[a]	0.442 ± 0.043[d]	0.705 ± 0.039[c]	0.408 ± 0.015[d]	0.874 ± 0.040[b]	0.072 ± 0.002[e]
malonylglycitin	0.112 ± 0.005[bc]	0.102 ± 0.013[c]	0.120 ± 0.004[ab]	0.124 ± 0.008[ab]	0.134 ± 0.005[a]	0.053 ± 0.014[d]
malonylgenistin	1.537 ± 0.039[a]	0.439 ± 0.038[d]	0.705 ± 0.074[c]	0.366 ± 0.01[e]	0.784 ± 0.034[b]	0.193 ± 0.004[f]
daidzein	0.023 ± 0.001[d]	0.037 ± 0.005[b]	0.028 ± 0.006[cd]	0.073 ± 0.002[a]	0.033 ± 0.004[bc]	0.031 ± 0.001[bc]
genistein	0.032 ± 0.001[a]	0.018 ± 0.006[b]	0.008 ± 0.001[c]	0.035 ± 0.004[a]	0.006 ± 0[c]	0.010 ± 0.001[c]

[a] Each value represents mean ± SD ($n = 3$); values in the same row with different superscript letters are significantly different ($p < 0.05$).

Table 2. Isoflavone Compositions of Soybeans of VS-KS 2 Cultivar Harvested from 2001 Fall, 2002 Spring, 2003 Fall, 2004 Spring, 2004 Fall, and 2005 Spring Crops

	isoflavone content (mg/g, freeze-dried weight)[a]					
composition	fall 2001	spring 2002	fall 2003	spring 2004	fall 2004	spring 2005
daidzin	0.982 ± 0.101[a]	0.204 ± 0.058[b]	0.204 ± 0.022[b]	0.171 ± 0.019[bc]	0.237 ± 0.081[b]	0.077 ± 0.031[c]
glycitin	0.053 ± 0.008[bc]	0.117 ± 0.032[a]	0.070 ± 0.014[b]	0.074 ± 0.009[b]	0.057 ± 0.017[bc]	0.031 ± 0.019[c]
genistin	2.593 ± 0.192[a]	0.569 ± 0.140[b]	0.479 ± 0.021[b]	0.379 ± 0.017[b]	0.601 ± 0.210[b]	0.016 ± 0.001[c]
malonyldaidzin	1.247 ± 0.073[a]	0.142 ± 0.039[c]	0.402 ± 0.021[b]	0.178 ± 0.010[c]	0.396 ± 0.030[b]	0.274 ± 0.175[bc]
malonylglycitin	0.061 ± 0.009[a]	0.056 ± 0.023[a]	0.065 ± 0.021[a]	0.051 ± 0.005[ab]	0.066 ± 0.008[a]	0.026 ± 0.004[b]
malonylgenistin	2.264 ± 0.185[a]	0.356 ± 0.144[c]	0.817 ± 0.046[b]	0.386 ± 0.006[c]	0.829 ± 0.079[b]	0.078 ± 0.024[d]
daidzein	0.015 ± 0.004[b]	0.012 ± 0.005[bc]	0.009 ± 0.001[c]	0.025 ± 0.002[a]	0.010 ± 0.001[c]	0.012 ± 0.001[bc]
genistein	0.024 ± 0.002[ab]	0.008 ± 0.009[de]	0.003 ± 0.001[e]	0.008 ± 0.001[cd]	0.019 ± 0.004[bc]	0.031 ± 0.002[a]

[a] Each value represents mean ± SD ($n = 3$); values in the same row with different superscript letters are significantly different ($p < 0.05$).

Table 3. Isoflavone Compositions of Soybeans of HBS Cultivar Harvested from 2001 Fall, 2002 Spring, 2003 Fall, 2004 Spring, 2004 Fall, and 2005 Spring Crops

	isoflavone content (mg/g freeze-dried weight)[a]					
composition	fall 2001	spring 2002	fall 2003	spring 2004	fall 2004	spring 2005
daidzin	0.605 ± 0.042[a]	0.235 ± 0.052[b]	0.279 ± 0.042[b]	0.278 ± 0.049[b]	0.264 ± 0.099[b]	0.065 ± 0.031[c]
glycitin	0.087 ± 0.007[a]	0.106 ± 0.026[a]	0.081 ± 0.006[a]	0.114 ± 0.023[a]	0.095 ± 0.039[a]	0.031 ± 0.033[b]
genistin	1.087 ± 0.025[a]	0.436 ± 0.059[b]	0.493 ± 0.036[b]	0.443 ± 0.023[b]	0.438 ± 0.130[b]	0.018 ± 0.003[c]
malonyldaidzin	0.652 ± 0.018[a]	0.235 ± 0.067[b]	0.598 ± 0.017[a]	0.257 ± 0.020[b]	0.594 ± 0.248[a]	0.091 ± 0.067[b]
malonylglycitin	0.073 ± 0.003[bc]	0.074 ± 0.029[bc]	0.107 ± 0.007[ab]	0.080 ± 0.015[b]	0.135 ± 0.064[a]	0.024 ± 0.007[c]
malonylgenistin	0.965 ± 0.085[a]	0.425 ± 0.145[bc]	0.845 ± 0.065[ab]	0.392 ± 0.039[c]	0.869 ± 0.346[a]	0.318 ± 0.464[c]
daidzein	0.006 ± 0.001[b]	0.009 ± 0.004[b]	0.009 ± 0.001[b]	0.028 ± 0.003[a]	0.006 ± 0.005[b]	0.011 ± 0.002[b]
genistein	0.007 ± 0.006[bc]	0.003 ± 0.001[c]	0.021 ± 0.001[a]	0.008 ± 0.001[bc]	0.020 ± 0.012[ab]	0.031 ± 0.008[a]

[a] Each value represents mean ± SD ($n = 3$); values in the same row with different superscript letters are significantly different ($p < 0.05$).

malonylgenistin, daidzein, and genistein contents of the tested cultivars are shown in **Tables 1**, **2**, and **3**. In general, daidzin, genistin, malonyldaidzin, and malonylgenistin were the majors and glycitin, malonylglycitin, daidzein, and genistein were the minors of soybean isoflavones. This was not in complete agreement with the observations of Fukutake et al. (*12*) who reported that genistin and daidzin constitute 99% of total isoflavones in the harvest soybean seeds. Chiou and Cheng (*9*) reported that genistin and daidzin are the major constituents of the detected isoflavones of soybeans extracted and analyzed with methanol–H_2O. For further investigation of the crop season effect, pairs of individual isoflavone contents on a successive year basis including 2001 fall/2002 spring, 2003 fall/2004 spring, and 2004 fall/2005 spring for each cultivar were compared. For the cultivar of CH 1 (**Table 1**), daidzin, genistin, malonyldaidzin, and malonylgenistin contents of the fall crops were all significantly higher than those of their spring crops ($p < 0.05$). For the cultivar of VS-KS (**Table 2**), only malonylgenistin contents of the fall crops were significantly higher than those of their spring crops ($p < 0.05$). For the cultivar of HBS (**Table 3**), malonyldaidzin and malonylgenistin contents of the fall crops were significantly higher than those of their spring crops ($p < 0.05$).

When isoflavone contents of each cultivar harvested from 3 years were integrated for statistical variance comparisons (**Table 4**), daidzin, genistin, malonyldaidzin, malonylglycitin, and malonylgenistin contents for each cultivar were significantly higher than those of their spring crops ($p < 0.05$). On the basis of the fact that these isoflavones were the major components, it is sure to state that soybean isoflavone contents were significantly higher in the fall crops than in the spring crops. In this study, since each cultivar of soybean was consecutively grown with rotation in the same experiment field, the reverse temperature profiles during soybean cultivation should have exhibited a dominant attribute to result in difference of isoflavone contents. When soybean plants with R5–R7 of maturity were respectively moved to growth chambers set at high and low temperatures until harvest (*7*), isoflavone contents of the soybeans harvested from low temperature are 14 to 16-fold higher than those

Table 4. Integrated Comparisons of the Compositional Isoflavone Contents of Soybeans Harvested from Cultivars of CH 1, VS-KS 2, and HBS Grown for 2001 Fall, 2002 Spring, 2003 Fall, 2004 Spring, 2004 Fall, and 2005 Spring Crops

		isoflavone content (mg/g, freeze-dried weight)[a]		
composition	season	CH1	VS-KS 2	HBS
daidzin	spring	$^{B}0.372 \pm 0.158^{a}$	$^{B}0.150 \pm 0.067^{b}$	$^{B}0.193 \pm 0.105^{b}$
	fall	$^{A}0.663 \pm 0.156^{a}$	$^{A}0.474 \pm 0.387^{ab}$	$^{A}0.383 \pm 0.177^{b}$
glycitin	spring	$^{A}0.143 \pm 0.035^{a}$	$^{A}0.073 \pm 0.041^{b}$	$^{A}0.084 \pm 0.046^{b}$
	fall	$^{A}0.115 \pm 0.028^{a}$	$^{A}0.091 \pm 0.033^{ab}$	$^{A}0.088 \pm 0.021^{b}$
genistin	spring	$^{B}0.407 \pm 0.182^{a}$	$^{B}0.321 \pm 0.253^{a}$	$^{B}0.299 \pm 0.213^{a}$
	fall	$^{A}0.796 \pm 0.331^{a}$	$^{A}1.225 \pm 1.038^{a}$	$^{A}0.672 \pm 0.319^{a}$
malonyldaidzin	spring	$^{B}0.307 \pm 0.178^{a}$	$^{B}0.198 \pm 0.107^{a}$	$^{B}0.194 \pm 0.092^{a}$
	fall	$^{A}1.035 \pm 0.360^{a}$	$^{A}0.682 \pm 0.426^{b}$	$^{A}0.614 \pm 0.128^{b}$
malonylglycitin	spring	$^{B}0.093 \pm 0.033^{a}$	$^{B}0.044 \pm 0.018^{b}$	$^{B}0.059 \pm 0.031^{b}$
	fall	$^{A}0.122 \pm 0.010^{a}$	$^{A}0.064 \pm 0.013^{a}$	$^{A}0.105 \pm 0.042^{b}$
malonylgenistin	spring	$^{B}0.333 \pm 0.111^{a}$	$^{B}0.273 \pm 0.164^{a}$	$^{B}0.378 \pm 0.248^{a}$
	fall	$^{A}1.009 \pm 0.400^{a}$	$^{A}1.303 \pm 0.728^{a}$	$^{A}0.893 \pm 0.188^{a}$
daidzein	spring	$^{A}0.047 \pm 0.020^{a}$	$^{A}0.016 \pm 0.010^{b}$	$^{A}0.016 \pm 0.009^{b}$
	fall	$^{B}0.028 \pm 0.006^{a}$	$^{A}0.012 \pm 0.005^{b}$	$^{B}0.007 \pm 0.003^{c}$
genistein	spring	$^{A}0.021 \pm 0.012^{a}$	$^{A}0.018 \pm 0.011^{a}$	$^{A}0.014 \pm 0.013^{a}$
	fall	$^{A}0.015 \pm 0.012^{a}$	$^{A}0.015 \pm 0.010^{a}$	$^{A}0.016 \pm 0.009^{a}$

[a] Each value represents mean ± SD ($n = 9$); values in the same column (A and B) for each isoflavone of each cultivar between spring and fall seasons with different superscript letters are significantly different ($p < 0.05$); values in the same row (a–c) for each isoflavone with different superscript letters are significantly different ($p < 0.05$).

harvested from high temperature. This was further demonstrated by Caldwell et al. (*13*) who cultivated soybeans in growth chambers with control of temperature, carbon dioxide, and moisture, and the higher the temperature, the lower isoflavone contents found. Lee et al. (*8*) successively grew soybeans for isoflavone analysis from 1998 to 2000 and concurrently recorded the ambient temperature during the last maturation periods. As observed, soybeans harvested in 1999 contained higher amount of isoflavones than the other two years and the temperatures of 1999 were only 1 °C lower than those of the other two years. In this study, soybeans were harvested in June and December for spring and fall crops and the difference of monthly temperature was up to 10 °C. There is no doubt that the observed difference of isoflavone content was mainly related to difference of the ambient temperatures between spring and fall crops.

As an extensive comparison of individual isoflavone contents was made among the test cultivars, each isoflavone content varied depending upon cultivar and crop season (**Table 4**). This was in agreement with the observation reported by Garrao-Panizzi and Kitamura (*14*) indicating that isoflavone contents vary in a wide spectrum as affected by cultivar (genotype) of Brazilian soybeans. This was also in agreement with the observation that isoflavone contents of soybeans vary considerably depending upon variety (*6*, *8*). Among the test cultivars grown for 3 years (**Table 4**), most isoflavone contents of CH 1 cultivar were significantly higher than those of either VS-KS 2 or HBS ($p < 0.05$). In accordance with the above observations and report of Lee et al. (*8*) that isoflavone contents of soybeans may vary considerably depending upon variety, location, growing season, climate, cultivation practice, and storage, it is apparent that biosynthesis of isoflavones of soybeans are driven both by their genotypes and phenotypes.

In conclusion, based on yield of extraction and HPLC resolution of soybean isoflavones, extraction with solvent of methanol–H_2O and HPLC analysis with acetic acid–acetonitrile as the mobile phase is suggested. As affected by crop season for each of the three cultivars grown for 3 years, daidzin, genistin, malonyldaidzin, and malonylgenistin contents were significantly higher for the soybeans of fall crops than their spring crops. From the viewpoint of soybean production to confer value-added health benefit of soybean isoflavones, the fall crop in Taiwan is suggested for mass soybean production.

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