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Study on Isoflavones Isomers Contents in Taiwan's Soybean and GM Soybean

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ABSTRACT

In this report we compared the total contents and the distribution of isoflavones in six soybeans, including 1 imported genetically modified (GM) soybean and 5 Taiwanese nongenetic modified soybeans, Koushing 10, Tainan 1, Tainan 4, Tainan 5 black, and commercial black. After the soybeans were soaked for 0, 4, 8, 12 and 16 hr in the solutions with different pH values (pH 3, 7 and 9), the contents of the isoflavones in the different 6 soybeans were measured. Nine isoflavones, including daidzin, genistin, and glycitin with a glucoside; acetyl-daidzin, acetyl-genistin, and acetyl-glycitin with acetylglucoside; and daidzein, genistein, and glycitein aglycones, were measured by HPLC. The total content of the isoflavones in Soybean Kaohsiung 10 was the highest at approximately 346 mg/100g. Soybean Tainan 5 black is the one with the lowest content of isoflavones. The isoflavones were present predominantly in forms with glucoside. After soaked in the water for different lengths of time, there was no significant loss of total isoflavones. In GM soybean, Koushing 10, and Tainan 5 black, the contents of aglycons, diadzein and genistein aglycones increased significantly after the soybeans were soaked in the water for 4 hr, compared with the ones in unsoaked soybeans ($P < 0.05$). In GM soybean, Koushing 10, and Tainan 5 black, the total contents of isoflavones in the soybeans soaked after 8 hr in pH 9 solutions were obviously lower than the ones in pH 3 and pH 7. The level of daidein and genistein from Kaohsiung 10 soaking in pH 3 was higher than pH 7 and pH 9. The contents of deidzein and genistein in Koushing 10 soaked in pH 3 solution was the highest, while the contents of deidzein and genistein in Tainan 5 black soybean soaked in pH 9 solution was the lowest.

Key words: non-GM soybean, GM soybean, isoflavones

INTRODUCTION

Isoflavones are composed of glycosides and aglycones. The aglycones, namely daidzein, genistein, and glycitein are generated via a hydrolysis process to remove the glucose under acid and alkaline conditions or by β-glucosidase(1). Isoflavones possess physiological regulation activities due to their chemical structure similarity to estrogen. Studies showed that isoflavones could possess the abilities to prevent breast, prostate, intestine, and stomach cancers(2,3), as well as osteoporosis by increasing bone density(4), and to reduce the low-density lipoprotein and cholesterol level in blood(5). Some epidemic investigations and studies revealed that American and English people have about four times more chances than Chinese and Japanese people to suffer from breast cancer, colon cancer, and prostate(6). Adlercreutz *et al*.(7) found that people who suffer less from hormone-related cancers (such as breast, prostate, and colon cancers) have higher concentration of phytestrogen (such as isoflavones) in urine. Messina(8) showed that western people (Canadian, American, English, French, and German) have three times higher risk in suffering breast and prostate than eastern people (Japanese and Hong Kong). That is because of the soybean dietary isoflavones, which support the prevention of cancers, osteoporosis, and cardiovascular diseases as well as reducing the menopause syndrome(9).

Many studies on isoflavones content in soybean are well documented. The isoflavones of soybean cultivated in different countries including America, Japan(10), Australia(11), Korea, and China(12) were analyzed. They were found to be similar in isoflavone compositions but varied in content depending on their genotype(13), maturity(14), time of harvest, and place of cultivation(15). The processed soybean contains both glycoside and aglycone isoflavones, which could be the result from the process of soaking, alkaline extraction, acid treatment, and the reaction of enzymes generated by microorganism during fermentation(1,16,17). The hydrolysis of β-glucoside bond enabling gentistin, daidzin and glycitine to be converted to gentisein, daidein, and glycitein, respectively, could affect the soybean isoflavone content and ratio(18,19). Daidzin and genistin are the two major isoflavones found in non-fermented soybean products; however, the aglycones, daidzein and genistein are the major isoflavones in fermented soybean products due to the enzymatic hydrolysis that occurs during the fermentation process(20).

Soybean (or yellow bean), *Glycine max* L. Merr., is a plant belonging to Glycime in Leguminosae. Soybean is an important source of vegetable protein and is the major raw material for making traditional oriental foods such as miso, natto, soy sauce, bean sprout, soymilk, and soy curd (Tou-

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Fu). Soybean comes in many different varieties. They can be categorized according to the fruiting period, the length of breeding time, and the color, type, and size of the seeds. In general, the shape of soybean seed is oval. The color of seed skin is milky yellow or black. The soybean with milky yellow color is called yellow bean; while the one with black skin is named black bean. The former has been used extensively as the raw material for soy curd and cooking bean, while the latter black bean is more suitable for cooking(1). In addition, some genetically modified (GM) soybean, such as Roundup Ready™ soybean (RRS) made in the USA, has been developed using modern biotechnology. Traditional soybean processing involves several steps in order to produce soy products. These include soaking, grinding and heating. Some processes involve microorganism fermentation and alkaline treatment to produce secondary by-products of different textures. Several researches regarding the isoflavones in soybean have been conducted. However, some areas of studies still need to be further explored. In this study, the differences in isoflavone content between the domestic non-GM soybean and imported GM soybean was investigated. The isoflavone content and its distribution in soybean under different soaking time with different pH of soaking solutions were also studied.

MATERIALS AND METHODS

I. *Materials and Reagents*

(I) *Materials*

GM soybean, Roundup Ready™ soybean (RRS) and non-GM soybean including Kaohsiung 10 soybean, Tainan 1 soybean, Tainan 4 soybean, and Tainan 5 black bean were provided by Tainan District Agriculture Research and Extrusion Station, Council of Agriculture, Executive Yuan. Marketed black beans were purchased from traditional markets.

(II) *Reagents*

Fluoresciein and the following isoflavone standards were purchased from Sigma Chem. Co. (USA): daidzin, genistin, daidzein, and genistein. Trifluoroacetic acid was obtained from Merck Co. (Germany). LC grade acetonitrile, methanol, and *n*-hexane were purchased from Tedia Co. (USA). Glycitin, acetyldaidzin, acetylglycitin, acetylgenistin, and glycitein standards were obtained from LC Laboratories (Woburn, MA, USA).

II. *Isoflavones Analysis*

(I) *Isoflavones extraction*

The extraction procedure used in this study was according to Kao[21]. Samples were freeze-dried and one gram of dried samples was weighed into a beaker. Ten milliliter of 80% methanol and 1 mL of internal standard were added into the beaker, which was then shaken at 60°C for 1 hr and centrifuged at 3,800 × g for 10 min. The upper layer was rotary-evaporated to dryness under vacuum pressure. The dried material was dissolved in 5 mL of 50% methanol and extracted 4 times with 20 mL of *n*-hexane. The methanol phase was then concentrated and 80% methanol was added to the residue to the final volume of 10 mL. The resultant solution was filtered through a 0.45 μm filter membrane prior to HPLC analysis.

(II) *HPLC analytical conditions*

The method published by Hautabarat[22] was adopted. A Hitachi HPLC system (Hitachi Ltd, Tokyo, Japan) equipped with a Model L-6200 pump, A Rheodyne Model 7125 injector, a Model L-4200 UV-VIS detector, and a Model D-2000 integrator was used in this study. A Licherichia RP-18 column (250 mm × 4.6 mm i.d., 5 μm) was used. Mobile phase consisted of (A) acetonitrile and (B) water containing 1% trifluoroacetic acid (v/v). A gradient programming was set as follows to deliver the mobile phase: starting from 10% (A) and 90% (B) for 10 min, linear gradient from 10% (A) to 55% (A) in 25 min, and then from 55% (A) to 10% (A) in another 5 min. The flow rate was 0.8 mL/min and injection volume was 5 μL. Nine isoflavones including daidzin, glycitin, genistin, acetyldaidzin, acetylglycitin, acetylgenistin, daidzein, glycitein, and genistein were then detected at 260 nm.

A. Preparation of standard solutions

a. Fluorescein used as an internal standard (1 mg/mL) was prepared in 80% methanol.
b. Isoflavone standard solutions: The standard solutions of daidzin, glycitin, genistin, acetyldaidzin, acetylglycitin, acetylgenistin, daidzein, glycitein, and genistein with different concentrations (0.5, 1.0, 5, 10, 20, 40, and 80 μg/mL) were prepared in 80% methanol of each containing internal standard fluorescienin (0.15 mg/mL).

B. Isoflavones quantitation

Isoflavones content = (peak area of isoflavones/peak area of internal standard) × (amount of internal standard/RR)/sample weight

Where RR (Relative response) = (peak area of internal standard × amount of isoflavones) / (peak area of isoflavones × amount of internal standard)

III. *Treatment with Different Soaking Time*

Soybean (25g) was weighed into a beaker and 100 mL of deionized water was then added. The soybean was soaked in water at 30°C for 0, 4, 8, 12 or 16 hr and then blended with a mixer at low temperature after soaking. The resulting mixture was then freeze-dried prior to extraction and HPLC analysis of isoflavones.

IV. *Treatment with Different pH Solution*

The samples (25 g for each) were weighed into a beaker separately. The buffer solutions (100 mL) with different pH (pH 3, 7, and 9) were added into each beaker containing soybean. The soybeans were soaked at 30°C for 8 hr, blended with a mixer, and freeze-dried. Isoflavones in soybean were then extracted and analyzed with HPLC. The buffer solutions of pH 3, 7, and 9 were prepared using citrate, deionized water, and sodium carbonate solutions, respectively.

V. *Statistical Analysis*

Each test was performed in triplicate. The data was analyzed with ANOVA variance analysis using Statistical Analysis System software(23) and Duncan's Multiple Range tests were used to carry out the difference analysis between treatments ($p < 0.05$).

RESULTS

I. *The Isoflavones Contents in Different Soybean Raw Materials*

The raw materials used in this study included non-GM soybeans: Kaohsiung 10, Tainan 1, and Tainan 4 soybeans with yellow seed skins, and Tainan 5 and commercial black beans with black seed skins, and imported Roundup Ready™ GM soybeans. The targeted isoflavones involved three categories based on their chemical structures. They were glucosides including daidzin, genistin, and glycitin; acetylglucosides including acetyldaidzin, acetylgenistin, and acetylglycitin; aglycones including daidzein, genistein, aglycitein. Table 1 lists the isoflavone content in soybeans. Total isoflavone contents in five tested non-GM soybeans were in the range of 59~346 mg/100g. Among them, the Kaohsiung 10 soybean contained the highest amount of total isoflavones (346 mg/100g). No significant difference of the total isoflavone content was found among Tainan 1 soybean, Tainan 4 soybean, commercial black bean, and GM soybean, total isoflavons were ranged at 130~170 mg/100g ($p > 0.05$). However, Tainan 5 soybean contained the least amount of total isoflavones (59 mg/100 g). HPLC chromatograms of isoflavones in Kaohsiung 10 soybean, Tainan 5 black bean, and GM soybean were shown in Figure 1. As shown in Table 1, except Tainan 5 soybean, which contained no glycitein, the other 5 soybeans all contained nine target analytes of isoflavones. The glycoside was the major isoflavone found in soybeans, which were especially rich in daidzin and genistin. It accounted for 93~96% of total isoflavones. The highest amount of daidzin and genistin was found in Kaohsiung 10

Table 1. Isoflavone content of five non-GM soybeans and GM soybean in Taiwan

Soybean	Isoflavone (mg/100 g)									
	Daidzin	Glycitin	Genistin	Acetyldaidzin	Acetylglycitin	Acetylgenistin	Daidzein	Glycitein	Genistein	Total
Kaohsiung 10	153.29^{a}*	30.20^{a}	137.02^{a}	7.52^{a}	1.84^{b}	5.07^{a}	7.51^{a}	0.03^{a}	4.31^{a}	346.80^{a}
	(9.73)**	(6.97)	(8.52)	(1.32)	(0.93)	(0.97)	(5.95)	(0.04)	(2.06)	(28.08)
Tainan 1	61.06^{bc}	11.26^{bc}	53.02^{b}	3.79^{b}	4.30^{ab}	2.78^{bc}	5.68^{a}	0.66^{a}	3.73^{ab}	146.28^{b}
	(11.63)	(1.10)	(13.88)	(1.79)	(0.03)	(0.98)	(1.59)	(0.75)	(0.04)	(30.20)
Tainan 4	51.76^{c}	23.61^{abc}	43.35^{b}	5.22^{ab}	8.05^{a}	3.19^{b}	4.25^{a}	0.64^{a}	2.42^{abc}	142.50^{b}
	(16.83)	(14.39)	(8.72)	(3.79)	(5.43)	(1.20)	(0.92)	(0.58)	(0.64)	(43.04)
Commerical	79.74^{b}	27.11^{ab}	51.18^{b}	5.09^{ab}	3.46^{ab}	1.24^{c}	0.44^{a}	ND***	1.44^{bc}	169.70^{b}
	(7.26)	(3.92)	(10.22)	(0.31)	(0.52)	(0.06)	(0.69)		(0.00)	(20.55)
Tainan 5	16.95^{d}	7.34^{c}	26.12^{b}	3.52^{b}	2.40^{b}	2.16^{bc}	0.48^{a}	ND	ND	58.99^{c}
	(7.26)	(3.92)	(10.22)	(0.31)	(0.52)	(0.06)	(0.69)			(20.55)
GM soybean	48.52^{c}	16.89^{abc}	47.60^{b}	3.63^{b}	5.00^{ab}	1.78^{bc}	3.68	0.33^{a}	2.94^{ab}	130.37^{bc}
	(7.80)	(3.47)	(6.34)	(0.49)	(0.54)	(0.17)	(0.63)	(0.29)	(0.56)	(19.12)

*Means followed by the same letter are not significantly different ($p < 0.05$).

**Standard deviation.

***ND, not detected.

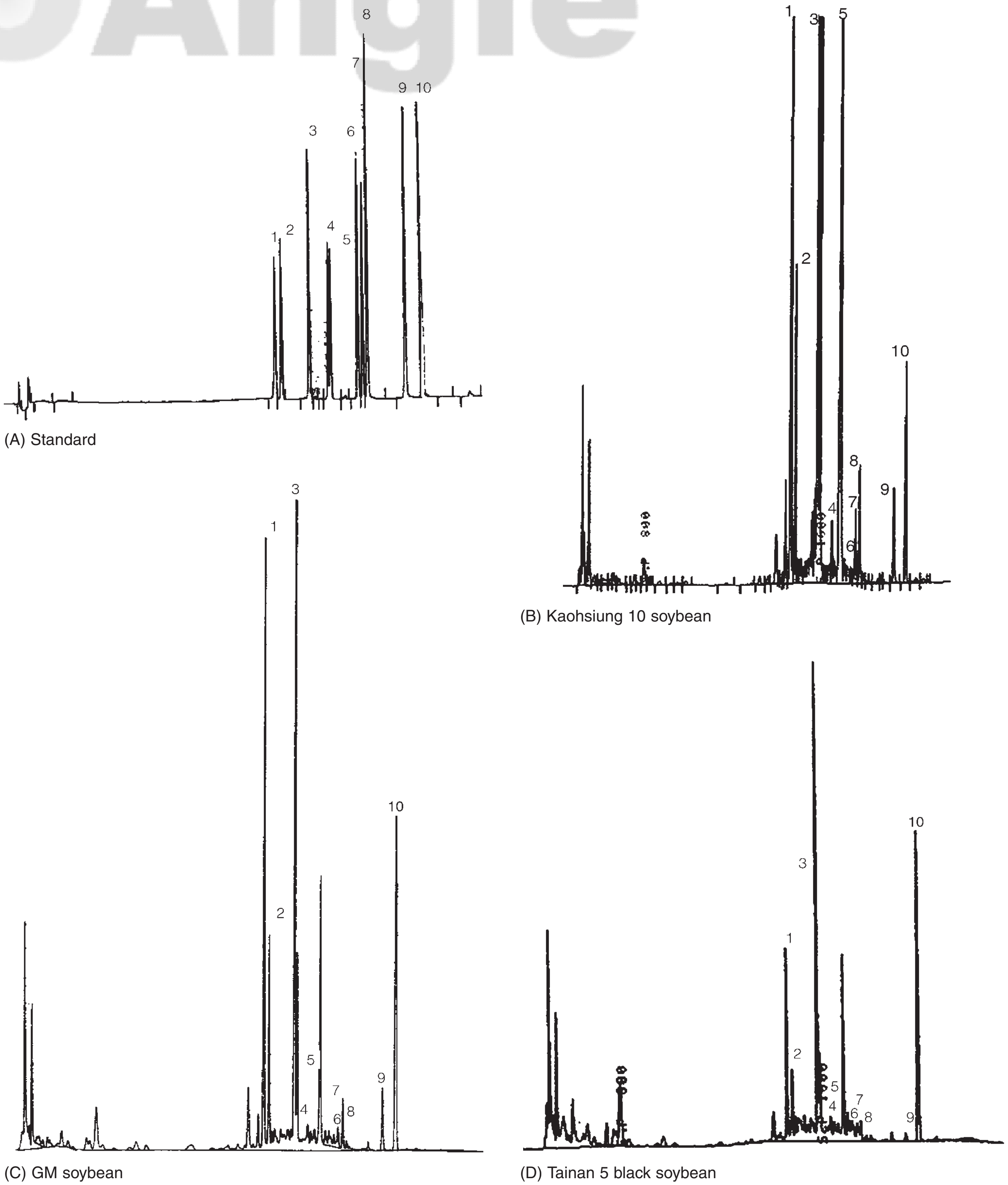

Figure 1. HPLC profile for isoflavones (A) standard (B) Kaohsiung 10 soybean (C) GM soybean (D) Tainan 5 black soybean.
The peaks were: 1. daidzin, 26.5 min, 2. glycitin, 27.1 min, 3. genistin, 29.6 min, 4. acetyldaidzin, 31.3 min, 5. acetylglycitin, 31.6 min, 6. acetylgenistin, 33.9 min, 7. daidzein, 34.4 min, 8. glycitein, 34.8 min, 9. genistein, 38.3 min, 10. fluoresciein, 39.7 min, respectively.

soybean. They were 153 and 137 mg/100g, respectively. Whereas, Tainan 5 black bean contained the least amount of daidzin and genistin (17 and 26 mg/100g, respectively). The above two isoflavones in GM soybean were 48 and 17 mg/100g, respectively. Acetyldaidzin, acetylgenistin, and acetylglycitin contents were in the range of 1~5 mg/100g; while aglycones daidzein, genistein, aglycitein were less than 7 mg/100 mg.

II. *Effect of Soaking Time on Isoflavone Content*

Soaking is routinely used for pretreatment processing to soften soybean raw materials. Three soybean raw materials, Tainan 10 soybean, Tainan 5 black bean, and GM soybean were used to study the soaking effect on isoflavone content. The above raw materials were soaked in water for the following time periods, 0, 4, 8, 12, and 16 hr and the isoflavone content with different soaking time were investigated. Results were shown in Table 2. No difference in total isoflavone content ($p > 0.05$) with increase of soaking time was found. While increasing soaking time could reduce daidein and genistin contents but glycitin, acetyldaidzin, acetylgenistin, and acetylglycitin content remained constant. Daidzein and genistein contents increased with increasing soaking time especially after soaking for more than 4 hr ($p < 0.05$). After soaking, the glucoside isoflavones in Kaohsiung 10 soybean decreased by 15~41 %, in Tainan 5 black bean, the amount decreased by 31~19 %, and in GM soybean , the amount decreased by 23~58 %. The daidzein and genistein contents in the above 3 soybeans were significantly increased ($p < 0.05$). In Kaohsiung 10 soybean, they were increased from 7.5 mg/100 g and 0.03 mg/100 g to 66.62 mg/100 g and 50.88 mg/100 g, respectively, after soaking for 8 hr. However, no significant difference was observed in Tainan 5 black bean after soaking for 8~16 hr. Daidzein and genistein slightly increased in amount from trace and not detected to 2.5~4.6 mg/100 g and 3.5~6.7 mg/100 g, respectively. While in GM soybean, daidzein and genistein increased from 3.7 mg/100 g and 3 mg/100 g, respectively to 33~35 mg/100 g for both after soaking for 12~16 hr.

III. *The Effect of Various pH Solutions on Isoflavone Content*

Table 3 lists the results for the changes of isoflavone content in Kaohsiung 10 soybean, GM soybean, and Tainan 5 black bean after soaking in pH 3, 7, or 9 solutions for 8 hr. As can be seen, except Kaohsiung 10 soybean, isoflavone content in Tainan 5 black bean and GM soybean was significantly lower in pH 9 soaking solution than in pH 3 and 7 solutions ($p < 0.05$). No significant difference in isoflavone content of Kaohsiung 10 and GM soybeans was found as soaked in pH 3 and 7 solutions ($p > 0.05$); while the isoflavone content of Tainan 5 black bean was lower in pH 3 soaking solution than in pH 7 solution. The changes in distribution of isoflavone isomers after 8 hr soaking are as follows. The content of glycosides including acetyldaidzin, acetylgenistin, and acetylglycitin was higher in pH 3 and 7 than in pH 9 soaking solutions. However, they were all less than 10 mg/100 g. The daidzein, glycitein, and genistein contents of Kaohsiung 10 soybean in pH 3 solution were 52.8, 4.82, and 38.84 mg/100 g, respectively, which were significantly higher than those in pH 7 and 9 solutions. They were 32.19, 1.54, and 25.62 mg/100 g, respectively, in pH 7 solution and 17.52, 0.51, and 10.87

Table 2. Isoflavone content of various soybean soaked by different time (hr)

Isoflavone	Content (mg/100 g)														
	Kaohsiung 10 soybean					GM soybean					Tainan 5 black				
	0	4	8	12	16	0	4	8	12	16	0	4	8	12	16
Daidzin	153.29^{a}*	118.14^{b}	68.21^{d}	54.01^{d}	99.72^{c}	48.52^{a}	41.33^{a}	26.01^{b}	14.39^{c}	20.38^{bc}	16.95^{a}	12.68^{a}	11.76^{a}	13.20^{a}	14.37^{a}
	(9.73)**	(5.15)	(7.27)	(18.42)	(9.12)	(7.80)	(4.40)	(2.69)	(2.19)	(7.26)	(1.30)	(3.05)	(0.73)	(6.67)	(2.36)
Glycitin	30.20^{a}	16.06^{b}	15.60^{b}	12.40^{b}	18.51^{b}	16.89^{ab}	17.88^{a}	12.96^{bc}	8.56^{d}	10.68^{cd}	7.34^{a}	4.48^{a}	6.12^{a}	4.26^{a}	7.22^{a}
	(6.97)	(5.76)	(4.40)	(4.13)	(1.66)	(3.47)	(3.20)	(0.43)	(0.48)	(1.43)	(3.92)	(4.50)	(3.04)	(3.86)	(5.15)
Genistin	137.02^{a}	112.49^{b}	78.01^{cd}	59.65^{d}	93.96^{c}	47.60^{a}	44.07^{a}	26.69^{b}	15.37^{c}	19.40^{bc}	26.12^{a}	16.53^{ab}	15.20^{b}	19.54^{ab}	18.43^{ab}
	(8.52)	(5.33)	(17.02)	(18.87)	(9.12)	(6.34)	(3.50)	(1.92)	(2.87)	(2.59)	(10.22)	(0.37)	(1.90)	(1.94)	(5.54)
Acetyldaidzin	7.52^{a}	5.06^{b}	4.48^{bc}	2.87^{c}	2.97^{bc}	3.63^{a}	2.16^{a}	2.87^{a}	2.99^{a}	2.80	3.52^{a}	3.96^{a}	3.37^{a}	3.53^{a}	2.28^{b}
	(1.32)	(1.32)	(1.09)	(1.29)	(0.94)	(0.49)	(0.45)	(0.33)	(0.39)	(0.08)	(0.31)	(0.06)	(0.58)	(0.13)	(0.15)
Acetylglycitin	1.84^{a}	3.06^{a}	2.59^{a}	2.15^{a}	2.77^{a}	5.00^{a}	3.86^{ab}	4.56^{ab}	3.46^{b}	4.02^{ab}	2.40^{a}	3.56^{a}	2.87	3.45^{a}	3.67^{a}
	(0.93)	(2.15)	(2.25)	(1.93)	(1.22)	(0.54)	(1.85)	(0.30)	(0.63)	(0.26)	(0.52)	(0.18)	(0.43)^{a}	(0.74)	(1.87)
Acetylgenistin	5.07^{a}	3.95^{b}	3.68^{b}	1.37^{c}	2.01^{c}	1.78^{a}	1.91^{a}	ND***	ND	ND	2.16^{a}	2.22^{a}	0.90^{a}	0.81^{a}	1.25^{a}
	(0.97)	(0.77)	(1.03)	(0.10)	(0.42)	(0.17)	(0.76)				(0.06)	(0.71)	(0.86)	(1.14)	(0.17)
Daidzein	7.51^{d}	32.19^{c}	66.62^{a}	54.79^{b}	40.56^{c}	3.68^{c}	21.44^{b}	24.59^{b}	35.57^{a}	35.95^{a}	0.48^{c}	1.12^{bc}	2.51^{abc}	3.41^{ab}	4.56^{a}
	(5.95)	(0.93)	(12.77)	(3.22)	(0.67)	(2.21)	(0.29)	(0.63)	(3.38)	(2.48)	(4.99)	(1.02)	(0.69)	(1.59)	(2.76)
Glycitein	0.03^{d}	1.54^{c}	5.21^{a}	4.69^{ab}	3.46^{b}	0.33^{c}	2.72^{b}	3.15^{a}	5.20^{a}	5.85^{a}	ND	0.29^{b}	ND**	ND	ND
	(0.04)	(1.35)	(1.19)	(0.53)	(0.76)	(0.29)	(0.41)	(0.29)	(0.79)	(0.62)		(0.42)			
Genistein	4.31^{e}	25.62^{d}	50.88^{a}	44.52^{b}	34.01^{c}	2.94^{c}	19.58^{b}	23.68^{ab}	34.14^{a}	32.99^{ab}	ND	2.22^{cd}	3.50^{bc}	5.46^{ab}	6.17^{a}
	(2.06)	(1.02)	(8.26)	(2.62)	(2.11)	(0.56)	(3.18)	(1.95)	(4.30)	(0.85		(0.05)	(0.87)	(0.01)	(2.33)
Total	346.80^{a}	318.12^{ab}	295.27^{bc}	236.44^{c}	297.97^{bc}	130.37^{a}	154.94^{a}	124.81^{a}	119.69^{a}	132.07^{a}	58.99^{a}	47.07^{a}	46.23^{a}	53.65^{a}	57.9 5^{a}

*Means followed by the same letter are not significantly different ($p < 0.05$). **Standard derivation. ***ND, not detected.

Table 3. Isoflavone content of various soybean which soaked in various pH

Isoflavone	Content (mg/100 g)								
	Kaohsiung 10 soybean			GM soybean			Tainan 5 black		
	3	7	9	3	7	9	3	7	9
Daidzin	135.82[a]*	118.14[a]	116.05[a]	29.79[b]	26.01[a]	7.01[c]	9.67[a]	11.76[a]	7.10[a]
	(20.09)**	(5.15)	(0.53)	(2.16)	(2.69)	(13.84)	(0.26)	(3.05)	(0.51)
Glycitin	27.98[a]	16.06[a]	18.84[a]	16.87[a]	12.96[a]	2.76[b]	5.17[a]	6.12[a]	3.58[a]
	(5.29)	(5.76)	(3.52)	(0.73)	(0.43)	(7.99)	(0.41)	(3.04)	(0.17)
Genistin	138.23[a]	112.49[a]	95.93[a]	32.89[b]	26.69[a]	8.82[c]	7.54[a]	15.20[a]	8.04[a]
	(15.28)	(5.33)	(1.71)	(3.33)	(1.92)	(12.97)	(10.66)	(1.90)	(1.45)
Acetyldaidzin	10.29[a]	5.06[a]	2.17[b]	4.02[a]	2.87[a]	ND***	2.83[a]	3.37[a]	1.38[b]
	(2.13)	(1.32)	(0.19)	(0.11)	(0.33)		(0.40)	(0.58)	(0.32)
Acetylglycitin	6.78[a]	3.06[b]	ND**	5.19[a]	4.56[a]	ND	2.17[a]	2.87[a]	ND
	(2.65)	(2.15)		(0.04)	(0.30)		(0.35)	(0.43)	
Acetylgenistin	8.31[a]	3.95[b]	ND	1.58[a]	ND	ND	1.92[a]	0.90[b]	ND
	(1.49)	(0.77)		(0.04)				(0.09)	(0.86)
Daidzein	52.80[a]	32.19[c]	17.52[b]	21.47[a]	24.59[b]	4.63[b]	1.19[a]	2.51[a]	0.88[a]
	(9.12)	(0.93)	(0.54)	(1.89)	(2.48)	(8.55)	(0.05)	(0.67)	(0.06)
Glycitein	4.82[a]	1.54[b]	0.51[b]	3.11[a]	3.15[b]	0.38[b]	ND***	ND	ND
	(2.14)	(1.35)	(0.10)	(0.51)	(0.29)	(0.95)			
Genistein	38.48[a]	25.62[c]	10.87[b]	20.59[a]	23.68[b]	3.03[b]	1.76[a]	3.50[a]	1.14[a]
	(7.32)	(1.02)	(0.60)	(1.20)	(1.95)	(5.76)	(0.13)	(0.87)	(0.04)
Total	412.61[a]	295.27[a]	295.25[a]	135.51[a]	124.81[a]	26.63[b]	32.24[a]	46.23[a]	11.60[a]

*Means followed by the same letter in the same are not significantly different ($p < 0.05$).
**Standard deviation.
***ND, not detected.

mg/100 g, respectively, in pH 9 solution. There was no significantly difference in daidezin, glycitein, and genistein contents of GM soybean soaked in pH 3 and 7 solutions. They were 21.47, 3.11, and 20.57 mg/100 g, respectively, in pH 3 solution, and 24.59, 3.15, and 23.68 mg/100 g, respectively, in pH 7 solution. However, the isoflavone content in pH 3 and 7 soaking solution was significantly higher than in pH 9 solution. Glycetein content of Kaohsiung 10 and GM soybeans in pH 3 solution was higher than that in pH 9 soaking solution. In pH 7 solution, the Tainan 5 black bean yielded higher daidein and genistein contents (2.51 and 3.50 mg/100 g, respectively) than in others. No glycitein was detected when Tainan 5 black bean was soaked in these 3 different pH solutions.

DISCUSSION

It has been reported that the isoflavone composition can be changed based on the variety of soybeans and growth environment[10,24,25]. Hoeck *et al.*[15] conducted a more than 2-year study on the effect of genotype, environment, and the interaction of gene and environment on isoflavone content of 6 varieties of soybeans. They concluded that the genotype was the most important factor. The isoflavone composition in 6 varieties of soybeans in this study is similar to the results of Wang and Murphy[10], who performed a study in analyzing the soybean raw materials and found that the isoflavone glycosides were the major component.

After soaking treatment, the isoflavone aglycones of Kaohsiung 10 soybean, Tainan 5 black bean, and imported GM soybean including daidein and genistein were likely to be formed. It is possible due to the hydrolysis of glycosides by β-glucosidase during soaking process resulting in the formation of aglycones[1]. Choi *et al.*[12] reported that daidein and genistein contents were increased after soaking soybean for 4 hr as compared to that without soaking treatment. Daidein and genistein contents of Kaohsiung 10 soybean, Tainan 5 black bean, non-GM soybean, and GM soybean were increased as soaking in acid solution. Wang and Murphy[26] found that the optimum condition for hydrolysis of isoflavone glycoside was in 1 N HCl solution at 98~100°C for 2 hr. Chiang *et al.*[27] utilized RSM study to optimize the acid hydrolysis condition that leaded to 97~100% isoflavones recovery. However, soaking in alkaline solution, the isoflavone contents of Kaohsiung 10 soybean, Tainan 5 black bean, non-GM soybean, and GM soybean were decreased. A study performed by Wang *et al.*[10] revealed that the total isoflavone content of alkaline-treated soybean was only 50% or less than that of Vinton soybean or its powder. This could be due to the changes of electric charges on isoflavone and protein under alkaline condition, leading to the reduction of the dissociation between isoflavone and protein. Daidzein, genistein, and glycitein contents of soybean were increased under alkaline condition. This could result from the hydrolysis reaction acted by β-glucosidase.

The absorption of isoflavone in human body starts from upper terminal of small intestine. Because of wide surface area, small intestine is capable of absorbing isoflavone efficiently[28]. However, the absorption efficiency to glycoside isoflavones is far less than that to aglycone isoflavones because of the larger molecular weight and more hydrophilic character of glycosides[29,30]. The composition and content of isoflavone are greatly affected by varieties of soybean, soaking time, and pH of soaking solution. The absorption efficiency of isoflavones is determined by their chemical structure. Aglycone isoflavones such as daidein and genistein can be absorbed in the upper terminal of small intestine. While glycoside isoflavones, daidin and genistin, which are the good substrates of β-glucosidase, can be hydrolyzed by β-glucosidase excreted by the microorganisms and absorbed in a form of chylomicrones. However, not all isoflavones are good enzyme substrates[5,31].

CONCLUSIONS

Soybean is an important source for obtaining alycone isoflavones which possess physiological activities. It is also a popular health food product that is currently welcomed by consumers. Our study showed that isoflavone content varied among varieties of soybean. The isoflavone content of imported GM soybean was no different from that of some domestic non-GM soybeans. However, Some domestic non-GM soybeans showed different as compared to the isoflavone content of imported GM soybean. In general, glycoside isoflavones are the major components. In terms of the changes of isoflavone content during soaking process with different soaking time and pH soaking solution, imported GM soybean, Kaohsiung 10 soybean, and Tainan 5 black bean showed the similar tendency of changes. The changes in glycoside and aglycone isoflavone contents could be due to the hydrolysis reaction acted by β-glucosidase during soybean processing. How to convert more glycoside into biologically active aglycone isoflavones will be the future approach of study.

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