Korean J. Crop Sci. 51(1): 81~88(2006)

Isoflavone Content and its Relationship with Other Seed Quality Traits of Soybean Cultivars Collected in South Korea

Sun-Lim Kim†, Hee-Youn Chi, Jung-Tae Kim, Yeong-Ho Lee, Nam-Kyu Park, Jong-Rok Son, and Si-Ju Kim

National Institute of Crop Science, R.D.A., Suwon 441-857, Korea

ABSTRACT: The 117 soybean cultivars were collected from nine provinces in Korea, and various seed quality traits along with isoflavone contents were evaluated to elucidate their relationship. The 100-seed weight of the black soybean (31.2 g) was significantly higher ($p < 0.05$) than yellow soybeans (28.6 g). The composition of genistein, daidzein, and glycitein accounted for 75.8, 22.8, and 1.4 % of total isoflavone in yellow soybean cultivars, while their compositions in black soybeans were 58.5, 39.7, and 1.8 %, respectively. The mean contents of total isoflavone in yellow and black soybean were 1,561.6 $\mu g\ g^{-1}$ and 1,018.3 $\mu g\ g^{-1}$. The isofalvone content showed significant variation among cultivars when classified by the seed size. In the yellow soybeans, total isoflavone content was higher in small size soybean cultivars (1,776.0 $\mu g\ g^{-1}$) and medium size soybean cultivars (1,714.3 $\mu g\ g^{-1}$) compared to large size ones (1,518.5 $\mu g\ g^{-1}$). Genistein content was proved as the major factor determining the relationship between isoflavone content and 100-seed weights (r = -0.206*). Daidzein and glycitein, however, showed no significant relationship with the 100-seed weights. Isoflavone content was not significantly correlated with color parameters L (lightness) and a (redness) values, but color parameter b (yellowness) was positively correlated with glycitein (r = 0.264*) in the yellow soybeans, while its negative correlation between daidzein (r= -0.245*) and total isoflavone (r = -0.256*) were observed in black soybeans. However, these findings suggested that the seed color value may not serve as an effective parameter for estimating the isoflavone intensity of the soybeans. Variation of protein and lipid contents between yellow soybeans (n = 58) and black soybeans (n = 59) was relatively stable, however, protein and lipid contents have no significant relationship with isoflavone content.

Keywords: soybean, protein, lipid, isoflavone, daidzein, glycitein, genistein

Soybeans (*Glycine max* [L.] Merr.) are a major source of vegetable protein and edible oil. The nutritional quality of soybean depends upon the relative abundance of specific proteins and fatty acids. Additionally, secondary metabolites such as isoflavones, in soybean seeds, have been shown to impact human health.

Soybean isoflavones are referred to as a phytoestrogen which has either weak estrogen-like or antiestrogenic activity (Fotsis *et al.*, 1993; Molteni *et al.*, 1995). Isoflavone is a group of naturally occurring heterocyclic phenols found mainly in soybean and the principal isoflavones of soybean seeds, such as daidzein, genistein, and glycitein are synthesized from the phenylpropanoid pathway and stored as glucoside conjugates in vacuole (Kudou *et al.*, 1991).

Isoflavones were reported to reduce the risk of breast cancer and heart disease through several mechanisms, and they are well-known to reduce total cholesterol levels and show some antioxidant activity (Anthony *et al.*, 1996; Barnes *et al.*, 1998; Molteni *et al.*, 1995).

Accumulation of isoflavone in soybean is cultivar-dependent and influenced by environmental conditions during the seed filling stage (Eldridge & Kwolek, 1983; Hoeck *et al.*, 2000; Kim *et al.*, 2004 & 2005c; Lee *et al.*, 2003; Tsukamoto *et al.*, 1995; Wang & Murphy, 1994).

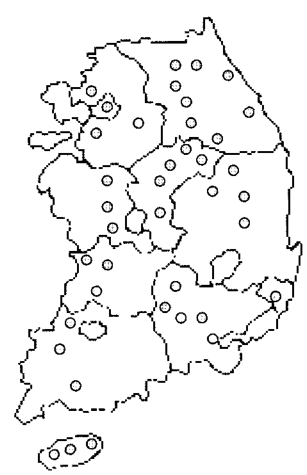
Recently, special emphasis has been given to the chemical composition of soybeans for improving various functional ingredients as well as for developing new soybean products and traditional soyfoods. Improvement of soybean components has been expected not only to improve food-processing properties but also to increase the nutritional quality of soybean products. Black soybeans that are consumed for cooking with rice, producing black soymilk, as a traditional medicine, and for many other purposes are treated as a special kind of soybean, and consequently are usually sold at a premium price in the market (Choung *et al.*, 2001; Espin *et al.*, 2000; Kim et al., 2005a & 2005c). Therefore, black soybeans currently receive much more attention as potential therapeutic agents against some pathological diseases.

However, little is known on the relationship of seed isoflavone content with various agronomic and other seed quality traits. The main objective of this study was to investigate whether isoflavone content in soybean seed is associated with changes in agronomic and seed quality traits by focusing. on the variations of isoflavones and their correlationship with major agronomic characteristics as well as with seed

†Corresponding author: (Phone) +82-31-290-6886 (E-mail) kimsl @rda.go.kr <Received March 9, 2006>

Table 1. Local collection of soybeans from the nine provinces in Korea.

Provinces	Seed color	
	Yellow	Black
Gyeonggi-do	7	5
Gangwon-do	17	13
Chungcheongnam-do	4	4
Chungcheongbuk-do	7	10
Gyeongsangnam-do	8	6
Gyeongsangbuk-do	5	8
Jeollanam-do	3	5
Jeollabuk-do	4	5
Jeju-do	3	3
	(n=58)	(n=59)



Each spot represents the regions of soybean collection in the nine provinces of Korea.

chemical components.

MATERIALS AND METHODS

Local collection of soybean

The 117 cultivars of soybeans were collected from the nine provinces in south Korea where slight ecological differences were observed. Table 1 represents the regions of soybean collection.

The collected soybeans were classified into two phenotypes: yellow and black soybeans based on their seed coat colors. Among the 117 collected soybeans, there were 58 yellow soybeans and 59 black soybeans. Soybean seeds were milled to flour and defatted with hexane by using an automatic fat extraction system (Gerhardt Soxtherm 2000, German) for the prepartion of isoflavone analysis.

Seed color evaluation

The Hunter's color value such as L (Lightness), a (Redness), and b (Yellowness) was measured by using color & color difference meter (Minolta Chromameter CR-200, Japan) which had been pre-adjusted with a standard white plate (L = 97.38, a = -0.02, b = 1.66).

Protein and lipid analysis

Soybean seeds were ground by using a laboratory test mill (Brabender, Germany) to produce about 100-mesh flour for the analyses of proteins, lipids, amino acids, and fatty acids. Protein content of seed sample was determined according to the Kjeldahl procedure using a Tecator Kjeltec Auto Analyzer (Model 2400, Foss Tecator, Sweden). Lipid content was measured by Soxtherm automatic system (Gerhardt, Germany). The extraction beakers were filled with a few boiling stones and then dried at 105 °C. The 5.0 g of homogenized sample was put into an extraction thimble and added with 140 ml of n-hexane. After boiling for 30 min at 180 °C, extraction was performed for 80 min with 5 times of solvent reduction. After extraction, the beakers were dried at 105 °C for 1 hour, then cooled down to room temperature in a desiccator and weighed. Total lipid contents were represented on a dry weight basis of soybean seeds.

Isoflavone analysis

One gram of defatted soybean flour was put into a test tube, suspended with 30 ml of 1 N HCl, and heated for 2 hrs at 100 °C with a reflux condenser in water bath. After digestion, the extract was volumed up to 100 ml with methanol and then the supernatant was filtered through a PTFE 0.45 μm syringe filter (Waters, Milford, MA, USA). The filtrate was injected for the HPLC analysis. Analysis of isoflavone was conducted by reverse-phase HPLC (Waters 2690 Alliance System, USA) equipped with a YMC-Pack ODS-AM303 (250 × 4.6 mm) connected with a guard column packed with μBonda C_{18} Waters guard-Pak pre column (Waters, Milford, MA, USA). The 0.1 % acetic acid in 35 % acetonitrile was employed for mobile phase. The solvent flow rate was 1.0 ml min^{-1} and following the injection of 20 ml of sample, the eluted isoflavones were detected at 254 nm by using a Waters 2487 dual λ absorbance detector. All HPLC analysis were performed at ambient temperature. The standard isoflavones were purchased from Sigma (St. Louis, MO, USA).

Statistical analysis

There were three replicates for all measurements. The data obtained from the analysis were statistically analyzed using SAS for Windows (Ver. 8.0, Statistical Analysis Systems Institute Inc., Raleigh, NC, USA).

RESULTS AND DISCUSSION

Seed characteristics

The 100-seed weights and color values of the local collection of 117 soybeans from the nine provinces in Korea were presented in Table 2.

The 100-seed weights of yellow soybean ranged from 24.6 g to 38.5 g, while the values of black soybean were 27.7~33.1 g. As compared with the mean values of 100-seed weights between yellow soybeans and black soybeans, black soybean (31.2 g) were significantly higher ($p < 0.05$) than yellow soybeans (28.6 g).

The color values of yellow soybean and black soybean were observed with considerable variations, although the seed coats of black soybeans have deep black color in their appearances. The L (lightness), a (redness), and b (yellowness) values of yellow soybean were ranged 49.8~63.9, 1.0~5.2, and 22.7~32.0, while the values of black soybean was 23.6~30.8, -0.3~3.2, and 0.6~1.5, respectively. This fact indicated that the color value is an important parameter for the evaluation of soybean seed characteristics.

The soybean seed colors determine the important quality traits, such as lustre, permeability, and nutritional value, and also affects visual appearance of soyfoods (Mullin & Xu, 2001; Kim *et al.*, 2005a & c). As soyfoods consumption

Table 2. Comparison on the 100-seed weights and color values between yellow- and black-soybeans collected from the nine provinces in Korea.

Seed color	Provinces	100 SW† (g)	Color values		
			L	a	b
Yellow soybean (n=58)	Gyeonggi-do	27.8± 9.4	54.6±3.1	1.6±3.2	22.7±3.8
	Gangwon-do	27.5± 8.6	61.8±4.2	2.6±3.6	28.9±2.8
	Chungcheongnam-do	24.6± 2.8	61.5±2.9	1.0±5.5	26.2±3.5
	Chungcheongbuk-do	30.5± 8.5	56.9±4.6	5.2±0.7	28.9±0.2
	Gyeongsangnam-do	26.9± 6.1	49.8±1.6	5.2±0.6	27.8±0.9
	Gyeongsangbuk-do	38.5±11.0	52.8±1.4	4.4±0.7	28.0±1.2
	Jeollanam-do	26.7± 1.8	60.3±0.7	3.5±0.0	32.0±0.0
	Jeollabuk-do	25.3± 2.8	63.9±1.7	4.0±0.7	31.0±1.6
	Jeju-do	30.8±10.3	63.3±1.6	4.7±1.0	29.9±1.5
	Mean±S.D.	28.6± 8.3	57.9±6.4	3.7±2.6	28.5±3.6
Black soybean (n=59)	Gyeonggi-do	27.7± 9.7	25.2±4.0	1.2±3.3	1.3±0.6
	Gangwon-do	30.0± 7.1	27.7±5.3	-0.3±0.5	1.1±0.6
	Chungcheongnam-do	33.1± 2.7	25.6±4.6	1.5±3.4	1.4±0.2
	Chungcheongbuk-do	31.2± 7.6	26.7±5.4	2.5±2.3	1.5±0.4
	Gyeongsangnam-do	31.3± 1.7	30.8±1.2	3.2±0.2	1.5±0.2
	Gyeongsangbuk-do	32.8±10.2	26.5±5.0	1.5±1.8	1.1±0.5
	Jeollanam-do	32.6± 4.2	23.6±4.3	-0.2±0.1	0.9±0.6
	Jeollabuk-do	32.5± 2.5	24.2±3.3	0.3±1.0	1.0±0.4
	Jeju-do	31.4± 5.1	23.6±2.9	-0.2±0.1	0.6±0.1
	Mean±S.D.	31.2± 6.6	26.4±4.6	1.1±2.1	1.2±0.5
LSD (≤ 0.05) between yellow & black soybean		2.52	2.04	0.86	0.91

†100 SW: 100-seed weight, L: lightness; a: (+) redness, (0) gray and (-) greenness; b: (+) yellowness, (0) gray and (-) blueness.

Table 3. Comparison on isoflavone content between yellow soybeans and black soybeans collected from the nine provinces in Korea.

Seed color	Provinces	Isoflavones (μg g^{-1})			
		Daidzein	Glycitein	Genistein	Total
Yellow soybean	Gyeonggi-do	349.5± 57.4	15.9± 8.4	1,293.3±261.6	1,658.6±300.1
	Gangwon-do	304.1±101.6	29.2±25.1	1,355.6±207.1	1,688.9±249.1
	Chungcheongnam-do	362.5±161.1	17.6±13.5	1,238.8±320.5	1,618.9±313.7
	Chungcheongbuk-do	441.2±212.3	18.5±13.0	1,303.2±161.2	1,762.9±352.8
	Gyeongsangnam-do	346.6±200.7	20.4± 6.5	1,168.0±101.7	1,535.1±200.1
	Gyeongsangbuk-do	480.3±163.1	12.1± 3.1	1,149.5± 83.4	1,641.9± 92.8
	Jeollanam-do	261.4± 14.9	41.9±19.7	1,134.1±139.3	1,437.4±151.9
	Jeollabuk-do	314.8± 42.3	20.8± 9.1	1,150.8± 49.9	1,486.5± 22.2
	Jeju-do	343.5± 70.4	19.7±11.7	958.2± 52.8	1,321.5±111.3
	Mean±S.D.	356.8±143.4	22.5±17.5	1,182.3±204.7	1,561.6±255.4
Black soybean	Gyeonggi-do	339.8± 59.5	19.4± 5.2	457.1±141.1	816.4±197.4
	Gangwon-do	451.9±108.1	17.9± 7.0	685.4±216.0	1,155.2±316.7
	Chungcheongnam-do	370.3± 78.5	16.6± 6.7	504.8±185.2	891.7±256.6
	Chungcheongbuk-do	363.5± 87.5	20.9± 8.1	588.4±245.2	972.8±312.7
	Gyeongsangnam-do	374.1±117.4	17.2± 8.1	560.9±205.7	952.2±322.6
	Gyeongsangbuk-do	391.7± 64.1	16.2± 3.5	590.5±159.0	998.4±221.1
	Jeollanam-do	383.4± 96.1	18.6± 6.4	549.6±151.3	951.5±249.0
	Jeollabuk-do	463.2± 57.5	17.0± 7.3	688.3± 66.2	1,168.4±123.7
	Jeju-do	497.5±111.4	17.7± 9.7	742.7±200.9	1,258.0±294.0
	Mean±S.D.	403.9± 95.0	17.9± 6.6	596.4±192.0	1,018.3±278.4
LSD ($p \leq 0.05$) between yellow & black soybean		ns†	ns	106.8	141.5

†ns: not significant.

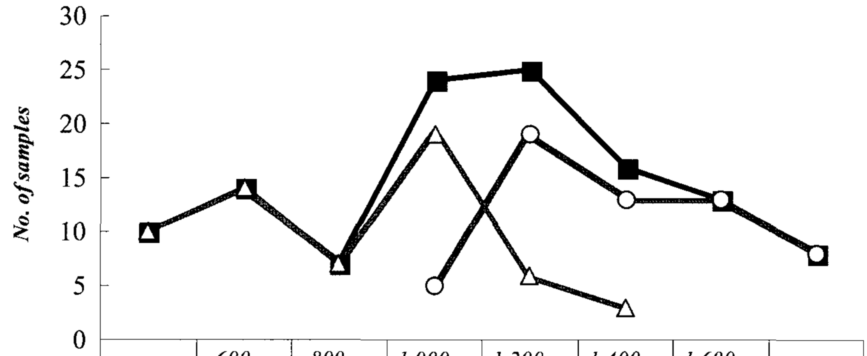
increases, colored soybeans are much more demanded for soy products such as colored soymilk, soycurd, and various traditional soy sources. Therefore, further accurate investigation of color parameter is necessary to evaluate the soybean color-related qualities.

Variation of isoflavone

It was surveyed in the present study whether seed colors have an effect on seed isoflavone levels. Isoflavone contents in the 58 yellow soybeans and 59 black soybeans collected in Korea are shown in Table 3.

Results showed that the most dominant isoflavone was genistein followed by daidzein and glycitein. Genistein, daidzein, and glycitein accounted for 75.8, 22.8 and 1.4 % of the total isoflavone in yellow soybeans, while their compositions in black soybeans were 58.5, 39.7, and 1.8%, respectively. The mean content of total isoflavone in yellow soybeans was 1,561 μg/g, while the content in black soybeans was 1,018 μg/g. The results indicated that yellow soybeans may accumulate isoflavone to higher levels than black soybeans. When the content of daidzein and glycitein were compared between yellow soybean and black soybeans, no statistically significan difference could be found in daidzein content, while glycitein content were higher in yellow soybeans. However, in case of genistein, its content was significantly higher in yellow soybeans, and the results suggested that the major differences of isoflavone content between yellow and black soybeans might be attributed to genistein level.

Fig. 1 represents the frequencies of collected soybeans as classified into 8 groups according to isoflavone contents. Out of 58 collected soybeans, the 53 yellow soybeans (corresponding to 89.8%) showed isoflavone content higher than 1,200 μg g^{-1}. In case of 59 black soybean, 50 soybeans (corresponding to 84.8%) showed isoflavone content lower than 1,200 μg g^{-1}. In this study, total 117 soybeans were collected from the nine provinces in Korea where ecological environment is slightly different. Consequently, our results supported previous reports that isoflavone content was affected



	< 600	600 ~ 800	800 ~ 1,000	1,000 ~ 1,200	1,200 ~ 1,400	1,400 ~ 1,600	1,600 ~ 1,800	> 1,800
Total (n=117)	10	14	7	24	25	16	13	8
Yellow soybean (n=58)				5	19	13	13	8
Black soybean (n=59)	10	14	7	19	6	3		

Isoflavone (ug g^{-1})

Fig. 1. Frequencies of isoflavone levels in the soybeans collected from the nine provinces in Korea.

Table 4. Variation of isoflavone content according to the seed size of soybeans collected from the nine provinces in Korea.

100-seed weight	Yellow soybean (n)	Daidzein	Glycitein	Genistein	Total	Black soybean (n)	Daidzein	Glycitein	Genistein	Total
			------- μg g^{-1} ------					------- μg g^{-1} ------		
15g >	2	356.7^{a†}	32.3^{a}	1,387.0^{a}	1,776.0^{a}	4	362.4^{a}	18.4^{a}	518.4^{a}	899.2^{a}
15-24g	14	370.9^{a}	19.5^{a}	1,323.8^{a}	1,714.3^{a}	0	-	-	-	-
24g <	42	344.8^{a}	23.3^{a}	1,213.4^{b}	1,581.5^{b}	55	405.3^{a}	18.1^{a}	607.0^{a}	1,030.4^{a}

†Means followed by common letters in a column are not significantly different at 5% level by DMRT.

by environmental factors such as temperature, cultivated location, as well as by genotype × environment interactions (Hoeck *et al.*, 2000; Kim *et al.*, 2005; Lee *et al.*, 2003). It was reported that the total isoflavone content varied from 1,160 to 3,090 μg g^{-1} among four soybean cultivars grown in the same environment, and from 460 to 1,950 μg g^{-1} across four locations (Eldridge & Kwolek, 1983). Futhermore, the total isoflavone content of a single soybean variety ranged from 1,176 to 3,309 μg g^{-1} among years and from 1,176 to 1,749 μg g^{-1} among locations within the same year (Wang & Murphy, 1994).

To elucidate the relationship between seed size and isoflavone content, the collected soybeans were classified into the three groups according to their 100 seed weight: small (< 15 g), medium (15-24 g), and large (24 g <) (Table 4). There was significant variation in isoflavone content according to the seed size of soybeans collected. In the yellow soybeans, total isoflavone content was higher in small (1776.0 μg g^{-1}) and medium size soybeans (1714.3 μg g^{-1}) than large size soybean (1518.5 μg g^{-1}). The variation in the level of genistein was considered as the major factor for the isoflavone differences among the 100-seed weights. In case of black soybeans, observed results were somewhat different compared to yellow soybeans, although they did not show the statistical differences. This results suggested that soybean isoflavone content, especially genistein, was variable and their physiological responses was different according to soybean seed colors. The biosynthesis of genistein has a different branch pathway from daidzein and glycitein, the availability of the substrate for certain isoflavone synthase relies on the activity level of a branch in phenylpropanoid pathway, and isoflavones are produced under certain environmental conditions where this branch pathway is active (Jung *et al.*, 2003).

The biosynthesis of isoflavone in black soybeans is assumed to have an interaction with anthocyanin which is abundant in black soybeans, and also being biosynthesized from the same phenylpropanoid pathway (Dixon & Paiva, 1995). So the special attention should be paid to study the relationship between anthocyanin and isoflavone synthesis in black soybean seed in the future.

Interaction of isoflavone with agronomic characteristics and chemical components

The relationship with 100-seed weight and isoflavones, daidzein, glycitein, and genistein are shown in Fig. 2. Weak but significant negative correlation (r = -0.206*) was observed between 100-seed weight and genistein content, while daidzein and glycitein were not significantly correlated with the 100-seed weights. This result was completely different to the report of Philippe *et al.* (2004), but such difference was considered mainly due to the physiological responses of black soybeans considerably different from those of yellow soybeans. Results of this study showed that the 100-seed weight of black soybeans was higher than those of yellow soybeans (Table 2), but isoflavone content of black soybeans were lower than yellow soybeans (Table 3). Therefore, negative correlation between 100-seed weight and isoflavone content can be explained by results.

The statistical relationship between the color parameters and the isoflavone content was measured by means of simple correlations (Table 5). The isoflavone content was not significantly correlated with Hunter's L and a values, but with b value. The correlationship, however, was dependent upon the seed coat color in that Hunter's b value was positively correlated with glycitein (r = 0.264*) in yellow soybeans, but negatively correlated with daidzein (r = -0.245*) and total isoflavone (r = -0.256*) in black soybeans. However, these results suggested that the color value is not an

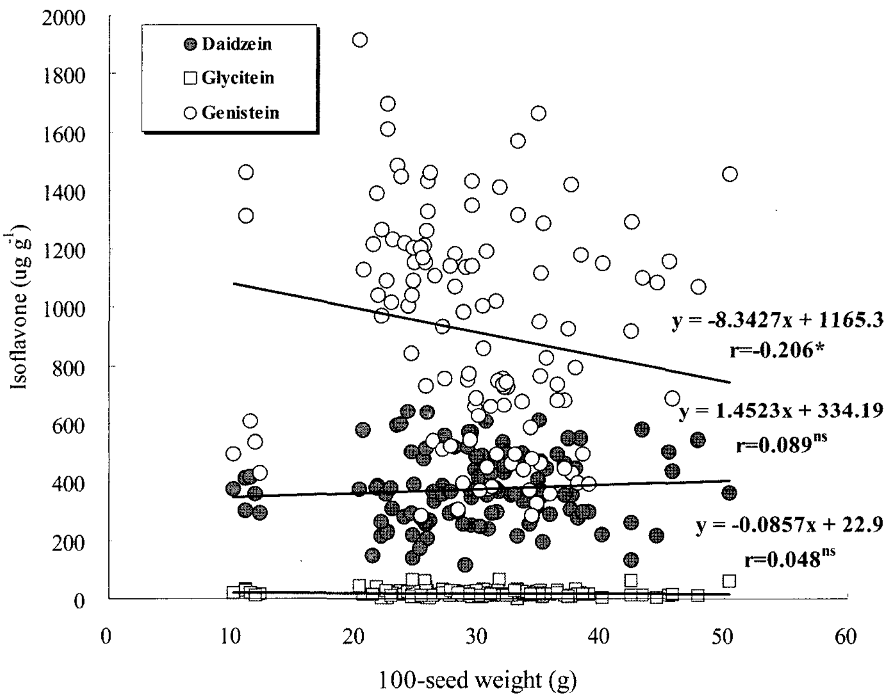


Fig. 2. Relationship with the 100-seed weights and isoflavones, daidzein, glycitein, and genistein of the collected soybeans in Korea.

Table 5. Correlation coefficients between color values and isoflavones in soybeans collected from the nine provinces in Korea.

Seed color	Color values	Isoflavones			
		Daidzein	Glycitein	Genistein	Total
Yellow soybean (n=58)	L	-0.016^{ns}	0.196^{ns}	-0.113^{ns}	-0.086^{ns}
	a	0154^{ns}	0.117^{ns}	-0.109^{ns}	0.007^{ns}
	b	0.062^{ns}	0.264*	-0.110^{ns}	-0.037^{ns}
Black soybean (n=59)	L	0.073^{ns}	-0.030^{ns}	0.125^{ns}	0.111^{ns}
	a	-0.128^{ns}	-0.007^{ns}	-0.042^{ns}	-0.073^{ns}
	b	-0.245*	0.053^{ns}	-0.196^{ns}	-0.256^{*}

ns: not significant, *significantly different at 5% level

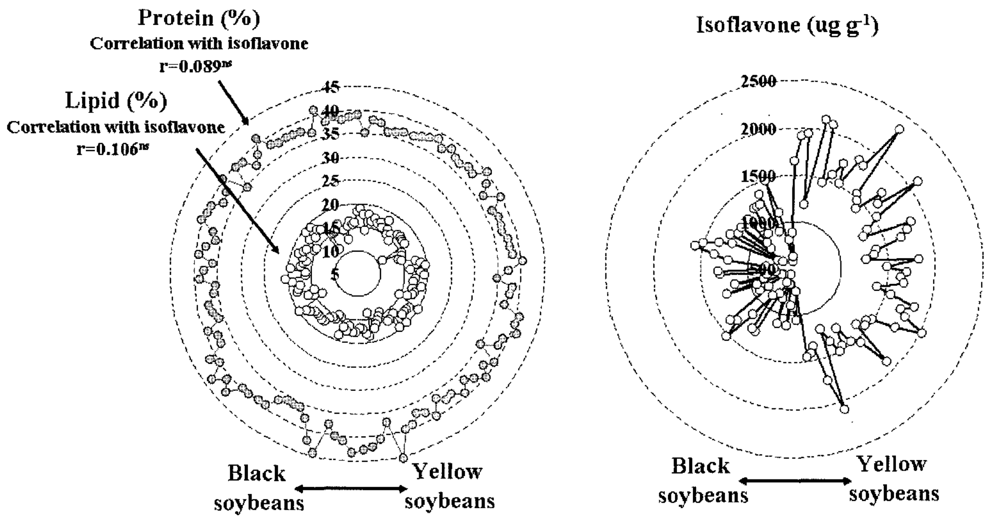


Fig. 3. Protein and lipid contents and their relationship with isoflavone content in soybeans collected from the nine provinces in Korea.

effective parameter for determining the isoflavone intensity of the soybeans.

Fig. 3 shows the variation plot of protein, lipid and total isoflavone contents of collected soybeans (n=117). Variation plot of protein and lipid contents between yellow soybeans (n=58) and black soybeans (n=59) was relatively stable within soybean samples and among soybean colors. However the variation plot of isoflavone content was relatively varied and the differences were high among soybean colors.

In general, protein and lipid composition often are influenced by genotype × environmental interactions such as growth temperature and plant nutrition (Gibson & Mullen, 1990). Environmental condition may alter the expression of genes in protein and lipid synthetic pathways in a manner that influences the composition and functional properties of protein, lipid, and isoflavone content which was reported as negatively correlated with protein (Philippe *et al.*, 2004). However our results showed that protein and lipid contents didn't have a significant correlation with isoflavone content. From the observation, we could suggest that this results were mainly due to the variations among soybean colors. But the exact reason responsible for the observed result remains obscure and needs further study. The understanding of biochemical and genetic mechanisms that regulate chemical components will lead us to develope new strategies for stabilizing protein, lipid, and isoflavone content.

REFERENCES

Anthony, M. S., T. B. Clarkson, C. L. Hughes, T. M. Morgan, and G. L. Burke. 1996. Soybean isoflavones improve cardiovascular risk factors without affecting the reproductive system of peripubertal rhesus monkeys. J. Nutr. 126 : 43-50.

Barnes, S. H. Kim, T.G. Peterson, and J. Xu. 1998. Isoflavones and cancer - the estrogen paradox. J. Kor. Soybean Dig. 15 : 81-93.

Choung, M. G., I. Y. Baek, S. T. Kang, W. Y. Han, D. C. Shin, H. P. Moon, and K. H. Kang. 2001. Isolation and determination of anthocyanins in seed coats of black soybean (*Glycine max* (L.) Merr.) J. Agric. Food Chem. 49(12) : 5848 -5851.

Dixon, R. A. and N. L. Paiva. 1995. Stress-induced phenylpropanoid metabolism. Plant Cell 7 : 1085-1097.

Eldridge, A. and W. Kwolek. 1983. Soybean isoflavones effect of environment and variety on composition. J. Agric. Food Chem. 31 : 394-396.

Espin, J. C., C. Soler-Rivas, H. J. Witchers, and C. Garcia-Viguera. 2001. Anthocyanin-based natural colorants: a new source of antiradical activity for foodstuff. J. Agric. Food Chem. 48 : 1588-1592.

Fotsis, T., M. Pepper, H. Adlercreutz, G. Fleischmann, T. Hase, R. Montesano, and L. Schweigerer. 1993. Genistein, a dietary-derived inhibitor of *in vitro* angiogenesis. Proc. Natl. Acad. Sci. 90 : 2690-2694.

Gibson, L.R. and R.E. Mullen. 1990. Soybean seed composition under high day night growth temperatures. J. Am. Oil Chem. Soc. 67 : 966-973.

Hoeck, J., W. Fehr, P. Murphy, and G. Welke. 2000. Influence of genotype and environment on isoflavone contents of soybean. Crop Sci. 40 : 48-51.

Jung, W. S., O. Yu, S. Lau, D. O'Keefe, J. Odell, G. Fader, and B. McGonigle. 2000. Identification and expression of isoflavone synthase, the key enzyme for biosynthesis of isoflavones in legumes. Nat. Biotechnol. 18 : 208-212.

Kim, J. J., S. H. Kim, S. J. Hahn, and I .M. Chung. 2005. Changing soybean isoflavone composition and contents under two different storage conditions over three years. Food Research International 38 : 435-444.

Kim, S. L., K. Y. Park, Y. H, Lee, and Y. H. Ryu. 2004. Seed quality of soybean produced from upland and drained-paddy field. Korean J. Crop Sci. 49(4) : 309-315.

Kim, S. L., H. B. Kim, H. Y. Chi, N. K. Park, J. R. Son, H. T. Yun, and S. J. Kim. 2005a. Variation of anthocyanins and isoflavones between yellow-cotyledon and green-cotyledon seeds of black soybean. Food Sci. Biotechnol. 14(6) : 778-782.

Kim, S. L., H. Y. Chi, J. R. Son, N. K. Park, and S. N Ryu. 2005b. Physicochemical characteristics of soybean seed coat and relationship to seed lustre. Korean J. Crop Sci. 50(S) : 291-300.

Kim, S. L., Y. H. Lee, H. T. Yun, J. K. Moon, K. Y. Park, and J. I. Chung. 2005c. Variation of chemical components and their interaction with isoflavones in maturing soybean seeds. Korean J. Crop Sci. 50(4) : 291-300.

Kudou, S., Y. Fleury, D. Welt, D. Magnolato, T. Uchida, and K. Kitamura. 1991. Malonyl isoflavone glycosides in soybeans seeds *Glycine max* (Merrill.). Agric. Biol. Chem. 55 : 2227-2233.

Lee, S. J., Y. Weikai, J. K. Ahn, and I. M. Chung. 2003. Effects of year, site, genotype and their interactions on various soybean isoflavones. Field Crop Res. 81 : 181-192.

Molteni, A., L. Brizio-Molteni, and V. Persky. 1995. In vitro hormonal effects of soybean isoflavones. J. Nutr. 125 : 751-756.

Mullin, W. J. and W. Xu. 2001. Study of soybean seed coat components and their relationship to water absorption. J. Agric. Food *Chem. 29 : 5331-5335.*

Philippe, S., Z. Wenju, L. S. Donald, and D. Wenhua. 2004. Isoflavone content of soybean cultivars grown in eastern Canada. Journal of the Science of Food and Agriculture 84(11) : 1327-1332.

Tsukamoto, C., S. Shimada, K. Igita, S. Kudou, M. Kokubun, K. Okubo, and K. Kitamura. 1995. Factors affecting isoflavone content in soybean seeds: Changes in isoflavones, saponins, and composition of fatty acids at different temperatures during seed development. J. Agric. Food Chem. 43 : 1184-1192.

Wang, H. and P. Murphy. 1994. Isoflavone composition of American and Japanese soybeans in Iowa: Effects of variety, crop year, and location. J. Agric. Food. Chem. 42 : 1674-1677.