Korean J Crop Sci 50(4) 291~300(2005)

Variation of Chemical Components and Their Interaction with Isoflavones in Maturing Soybean Seeds

Sun-Lim Kim*†, Young-Ho Lee*, Hong-Tae Yun*, Jung-Kyung Moon*, Keum-Yong Park, and Jong-Il Chung*****

*National Institute of Crop Science, R D A , Suwon 441-857, Korea
**Yeongnam Agricultural Reserarch Institute, National Institute of Crop Science, R D A , Milyang, 627-803, Korea
***College of Agriculture and Life Sci , Gyeongsang Natl University, Jinju 660-701, Korea

ABSTRACT: This study was focuses on the variation of isoflavone contents during seed development and their interaction with major chemical components such as protein, amino acids, saccaharides, lipid and fatty acids. During maturing, lipid, protein, and amino acid contents in soybean seeds showed the highest values at R7 stages, but isoflavone contents were increased until R8 stage. It was noted that malonyl glucosides (64.2%) are predominant forms among conjugated isoflavones followed by glucosides (30.7%), acetyl glucosides (4.1%) and aglycones (0.9%). Sucrose and stachyose were presented as a major saccharide in soybean seeds. As maturing days progressed, they were constantly increased and the highest contents were observed at R8 stage. While small quantities of raffinose, fructose, glucose, maltose, DP3 (DP: degree of polymerization), DP6, and DP7 were detected. These results showed that saccharide composition at the beginning of seed development is primarily monosaccharides with little sucrose and oligosaccharides, but as maturing days proceeds, sucrose and starch increase with concomitant decrease in monosaccharides. Sucrose and stachyose were positively correlated with isoflavone (r=0.780, 0.764 at p<0.01, respectively), while fructose, glucose, maltose, and DP7 were negatively correlated (r=-0.651, -0.653, -0.602, and -0.586 at p<0.05, respectively). Soybeans at R8 stage were high in protein and amino acid, but low in free amino acid contents. Protein and amino acid contents showed positively significant correlations with isoflavone (r=0.571 and 0.599 at p<0.05, respectively), but free amino acid content were negatively correlation with isoflavone (r=-0.673, p<0.01). The lipid content reaches its final content relatively early stage of seed development and remains constant as compared with other chemical components. Among the fatty acids, although varietal difference was presented, stearic acid and linolenic acid were gradually decreased, while oleic and linoleic acid were increased as seed maturing progressed. Lipid was significantly correlated (r=0.754, p<0.01) with isoflavones. However, neither saturated fatty acid nor unsaturated fatty acids significantly affected the isoflavone contents of maturing soybean seeds.

†Corresponding author (Phone) +82-31-290-6886 (E-mail) kimsl@rda go.kr
<Received September 1, 2005>

Keywords: soybean, protein, amino acids, lipid, fatty acids, saccharide, isoflavone, reproductive stage

Soybeans (*Glycine max* [L.] Merr.) are a major source of vegetable protein and oil, and thus has become one of the most valuable of cultivated crops. The nutritional quality of these seed components depends upon the relative abundance of specific proteins and fatty acids. Enhancing nutritional value of seed protein involves increasing total protein, enhancing content of particular subunits, increasing specific amino acids, and minimizing the proteins that have been shown to have deleterious effects. The relative content of fatty acids influences the physical and chemical characteristics of the oil and the suitability of the oil for a particular use. Soybean lines are currently being developed to express amended fatty acids increasing potential uses of the oil (Kinney, 1996; Wilson *et al.*, 2001; Spencer *et al.*, 2004).

Mature soybean seeds contain 6-17% of soluble carbohydrates. The majority of the carbohydrates are oligosaccharides, including sucrose and the raffinose series (Hymowitze & Collins, 1974; Kawamura & Tada, 1967), determination of the composition and metabolism of soluble oligosaccharides during seed development will provide a essential information for the improvement of soybean seed quality.

Sugars are important in all organisms, both as carriers of stored chemical energy and as raw materials for the synthesis of other molecules during all stages of the plant life cycle. Therefore, sugar concentrations affect the expression of a large number of genes and other chemical compounds (Koch, 1996).

In addition to the major seed storage compounds, soybean also contains isoflavone which serves a variety of biological functions. Isoflavone is a group of naturally occurring heterocyclic phenols found mainly in soybean and has been credited with performing several health-promoting functions. Several investigators have suggested that soy food consumption may contribute to lower rates of chronic diseases such as hormone-dependent cancers, cardiovascular

diseases, and osteoporosis (Anderson *et al.*, 1995; Anthony *et al.*, 1996; Barnes *et al.*, 1998; Schultz, 1998). The principal isoflavones of soybean seeds, daidzein, genistein, and glycitein, are synthesized from the phenylpropanoid pathway and stored as glucoside conjugates in vacuole (Kudou *et al.*, 1991).

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

This study focuses on the changes of isoflavone contents during seed development and their interaction with major chemical components such as protein, amino acids including free amino acids, saccaharides, lipids and fatty acids.

MATERIALS AND METHODS

Soybean cultivars

Three Korean soybean cultivars 'Sowonkong', 'Sinpaldalkong 2', and 'Hanagarikong' were grown and harvested at the field of National Institute of Crop Science, Suwon, Korea in 2003. The chemical characteristics of experiment soil was pH 6.7, O.M. 1.5%, available P_2O_5 338 ppm, exchangeable cation K, Ca, and Mg were 0.49, 2.3, and 1.2 me/100g, respectively. For the analysis of nutrients in soybean seeds during the reproductive stages, soybean seeds were sampled from the R5 through the R8 stages, and seeds of R5 and R6 stages freeze dried immediately after sampling and stored at 4 °C until analysis.

Protein and lipid analysis

Soybean seeds were ground by using laboratory test mill (Brabender, Germany) about 100 mesh flour for the analysis of proteins, lipids, amino acids, and fatty acids. Protein content of seed samples 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 beaker was filled with a few boiling stones and then dried at 105 °C. The 5.0 g of homogenized sample was put into extraction thimble and add 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 basis of soybean seeds.

Amino acid analysis

Each 0.3 g sample of soybean seed was weighed and 5 ml of 6 N-HCl was added. The hydrolysis was maintained for 24 h at 110 °C in test tubes with nitrogen gas flushing. Afterwards, the samples were diluted to the 100 ml of Milli-Q water and filtered with Millipore 0.45 μm- syringe filters (Milford, USA). The 1 ml of each hydrolysate was put into an autosampler bottle and injected into an amino acid autoanalyzer (Hitachi L-8800, Japan). The amount of each amino acid present in the samples was calculated with reference to the standard amino acids (Ajnomoto-Takara Co., Japan).

Free amino acid analysis

Free amino acid (FAA) contents were determined by L-8800 high-speed amino acid analyzer. The 1.0 g of soybean sample was diluted with 10 m*l* of 3% trichloroacetic acid solution. The sample was left at the room temperature for 1 h, centrifuged at 10,000 g for 15 min. The collected supernatant was filtered with Millipore 0.45 μm syringe filters. The filtrate was loaded on amino acid analyzer. The standard amino acid solutions, Type AN-II and Type B, were obtained from Wako (Wako-shi, Japan).

Mono- and oligo-saccaharide analysis

Mono- and oligo-saccaharides were analyzed using YMC-Pack Polyamine II column 5 mm (4.6 × 250 mm) from YMC Co., Ltd (Kyotyo, Japan). Twenty milliliters of 20% ethanol solution was added to 1.0 g of sample and shaken for 60 min at 35 °C water-bath. It was then centrifuged at 5000 g for 5 min. The collected supernatant was filtered with Sep-Pak NH_2 solidphase extraction cartridge (Waters, USA), then 1.0 ml of filtrate was evaporated to dryness at 50 °C dry-bath by blowing with N_2 gas. The residue was dissolved in 0.2 ml of water. Then 20 μ*l* was injected into HPLC equipped with Waters 510 Pump, Waters 410 R.I. Detector, and Waters 746 Integrator. The operating conditions were as follows: column temperature 35 °C; detector temperature 39 °C; mobile phase acetonitrile: water (65 : 35, v/v); flow rate 0.7 ml min^{-1}. The mono- and oligo-saccharides were obtained from Sigma (St. Louis, USA).

Fatty acid analysis

The fatty acids were analyzed by Rafael and Mancha's method (1993). The procedure was as follows. The 0.5 g of soybean flour was heated with a reagent containing methanol : heptane : benzene : 2,2-dimethoxypropane : H_2SO_4 =

37 : 36 : 20 : 5 : 2 (v/v).

The simultaneous digestion and lipid transmethylation were taken place in a single phase at 80 °C. After cooling at room temperature, the upper phase containing the fatty acid methyl ester (FAME) was prepared for the capillary GC analysis. The GC analysis was performed on a Agilent 6890 system (HP Co., U.S.A.) equipped with a FID by using a HP-Innowax capillary 30 m × 0.25 mm × 0.25 μm film (Cross-linked polyethylene glycol) column. The initial temperature of 150 °C was increased to the final temperature of 280 °C at the rate of 4 °C/min. Carrier gas was nitrogen at the flow rate of 10 ml min^{-1}. During the analysis, the temperatures of inlet and detector were maintained to 250 and 300 °C, respectively. The standard FAME Mix (C_{14}-C_{22}) was purchased from Supelco (USA).

Isoflavone analysis

For the analysis of the 12 isoflavones, the 0.5 g of freeze-dried and finely ground soybean was weighed into a test tube and 10 ml of 50% acetonitrile was added to each flask. The solution was mixed using a vortex mixer and overnight at room temperature. Extracts were suction filtered through Whatman No.42 filter paper and washed twice with 10 ml volumes of extraction solution. Samples were condensed to approximately 1 ml using a vacuum rotary evaporator (Labo Rota S-300, Resona, Switzerland) with a water bath at 30 °C. The dried material was re-dissolved in a mixture of methanol:water (80 : 20, v/v) to a final volume of 3 ml and filtered through a 0.45 μm PTFE syringe filter (Waters, Milford, MA, USA) prior to HPLC analysis. The filtrate was injected for the HPLC analysis. Analysis of isoflavone was conducted by reverse-phase HPLC equipped with YMC-Pack ODS-AM303 (4.6 × 250 mm) (YMC Inc, Wilmington, NC) connecting a guard column packed with μBonda C_{18} Waters guard-Pak pre column (Waters, Milford, MA, USA) The mobile phases for HPLC consisted of solvent (A) 0.1% acetic acid, and (B) 0.1% acetic acid in acetonitrile. The solvent gradient was as follows: Solvent B was increased from 15 to 25% over 35 min, then increased to 26.5% within next 12 min, and finally to 50% within 50 s and held at that percentage for next 14.5 min. The flow rate was 1.0 ml min^{-1} up to 48 min and increased to 1.3 ml min^{-1} from 48.5 min till 63 min. The injection was 20 μl of sample and the eluted isoflavone were detected at 254 nm by using Waters 2487 dual λ absorbance detector. All HPLC analysis were performed at ambient temperature.

The 12 isoflavone standards, three aglycones such as daidzein, genistein, and glycitein, and three glucosides such as daidzin, genistin, glycitin were purchased from Fujicco Co., LTD (Kobe, Japan). Three malonylglucosides such as malonyldaidzin, malonylgenistin, and malonylglycitin, and three acetylglucosides such as acetyldaidzin, acetylgenistin, and acetylglycitin were purchased from Wako pure chemical Industeries, Ltd (Osaka, Japan) and PKC Pharmaceuticals, Inc. (Woburn, MA, USA), respectively.

Statistical analysis

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

RESULTS AND DISCUSSION

Characteristics of seed development

Seed length, width, and length/width (L/W) ratio were surveyed to define the development patterns of soybean seed (Fig. 1 and 2).

In general, soybean seeds begin to growth when soybean plants are reached at R5 stage, and at this stage the seed sizes are approximately 3 mm long. At R6 stage soybean seeds are in the state of full seed-pod containing green seed filled up the pod cavity. When soybean seeds are reached at R7 stage, seeds commence to mature and some pods are physiologically matured. At final R8 stage, soybean seeds are full matured and more than 95% of the pods showed matured pod color.

In this study, we observed the length, width, and length/width ratio (L/W) of seed were relatively uniform within the same variety but varied distinctly among the cultivars. The seed length of three soybean cultivars was increased from R5 stage through R7 stage, and decreased slightly by R8 stage. While the seed width of three soybean cultivars was increased until R8 stage.

Length/width ratio (L/W) values were ranged from 1.03 (R8 stage of 'Hanagarikong') to 1.47 (R6 stage of 'Sowonkong'), and the highest L/W values were observed at R6 stage and rapidly decreased until R8 stage of all soybean cultivars. As compared with the L/W values among soybean cultivars, the changing degree of 'Sowonkong' (1.47-1.05) was higher than those of 'Sinpaldalkong 2' (1.33-1.17) and 'Hanagarikong' (1.24-1.03).

The L/W values represent the shape of soybean seeds and visually scored as 1 for round to 1< for oblong seed. As seed maturing days progressed, L/W values of 'Sowonkong' and 'Hanagarikong' were sharply decreased, while the values of 'Sinpaldalkong 2' were relatively high because of the developing degree of seed width was lower than those of two cultivars. The green soybeans turned yellow at R7 stage (40-45

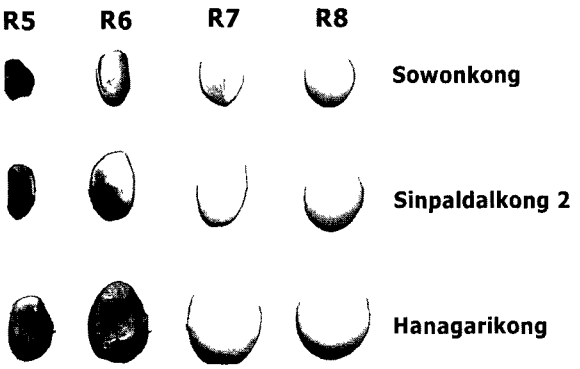

Fig. 1. Profile of soybean seed development at R5, R6, R7, and R8 stages Seeds of R5 and R6 stages were freeze dried immediately after sampling.

days after flowing) (Fig. 1).

A decrease in seed moisture content continues at a faster rate until the later stage of maturation, accompanied by an increase in seed dry weight. However, when seed weight is expressed on a dry basis, it increases rapidly from R5 through R7 stage, and decreased slightly by R8 stage (Fig. 2).

Isoflavone

The accumulation of isoflavone daidzein, genistein, and glycitein, and their corresponding acetylated and malnoylated forms in R5 ~ R8 stage of maturing soybean seeds were shown in Fig. 3, 4, and Table 1.

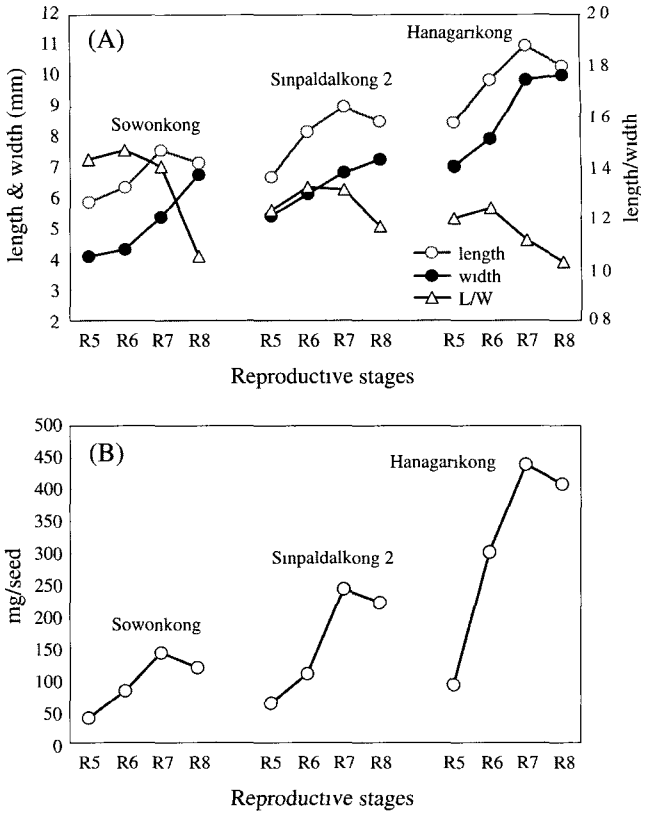

Fig. 2. Changes of length, width, length/width ratio (A), and seed dry weight (B) during reproductive stages R5, R6, R7, and R8 stage of Korean soybean cultivars, 'Sowonkong', 'Sinpaldalkong 2', and 'Hanagarikong'

Table 1. Isoflavone contents in Korean soybean cultivars, 'Sowonkong', 'Sinpaldalkong 2', and 'Hanagarikong' during the reproductive stages R5, R6, R7, and R8 stages

Cultivars	RS†	aglycones			glucoside			malonyl glucoside			acetyl glucoside			total
		Dai[1]	Gen[2]	Gly[3]	Dai[4]	Gen[5]	Gly[6]	Dai[7]	Gen[8]	Gly[9]	Dai[10]	Gen[11]	Gly[12]	
		μg/g												
Sowonkong	R5	4.4	3.9	3 7	42.1	42 1	50.1	111.2	130.4	56.9	24 2	5.5	-	474.4
	R6	4.0	4.1	3.0	130.5	239.2	127.2	315 5	580 7	117.5	52.2	5.7	-	1579.6
	R7	4.2	3 7	3 2	256 8	513.5	113.7	555.8	1110.9	114 9	85.4	7.4	-	2769.4
	R8	3.7	3.7	2.0	336.8	589.3	111.0	755.8	1391.4	113.7	94.9	6 5	-	3408.7
Sinpaldalkong 2	R5	6 8	6.3	6.0	70.2	70.2	80.7	179 3	210.4	86.2	39.0	8.8	3 6	767.4
	R6	6.2	6 6	4 9	210 5	385.8	201 8	394.4	841.6	178.0	84.2	9.2	3.1	2323 2
	R7	5.0	5.9	5.1	421.0	841.8	174.9	896.4	1542.9	143.6	137.7	18 6	-	4195.9
	R8	6.2	5.9	3.2	561.3	982.1	168.2	1219.1	2244.2	172.3	153.0	9.3	-	5524.8
Hanagarikong	R5	2.7	2.4	2.2	25.3	33.7	36.0	77.8	104.4	45 5	19.4	3.4	-	352 6
	R6	2.6	2.0	2 1	80 9	186 6	80.1	268.2	400.7	77 6	32.3	3.5	-	1136.7
	R7	2 9	2.3	2.0	156.6	313.2	91.0	344.6	799.8	91.9	52.9	3.7	-	1860.9
	R8	2.4	2 3	2.2	202 1	353 6	99 9	468.6	862 7	75.1	58.8	4.6	-	2134 8

†*RS Reproductive stage 1, daidzein, 2, genistein, 3, glycitein, 4, daidzin, 5, genistin, 6, glycitin, 7, malonyldaidzin, 8, malonylgenistin, 9, malonylglycitin, 10, acetyldaidzin, 11, acetylgenistin, 12, acetylglycitin*

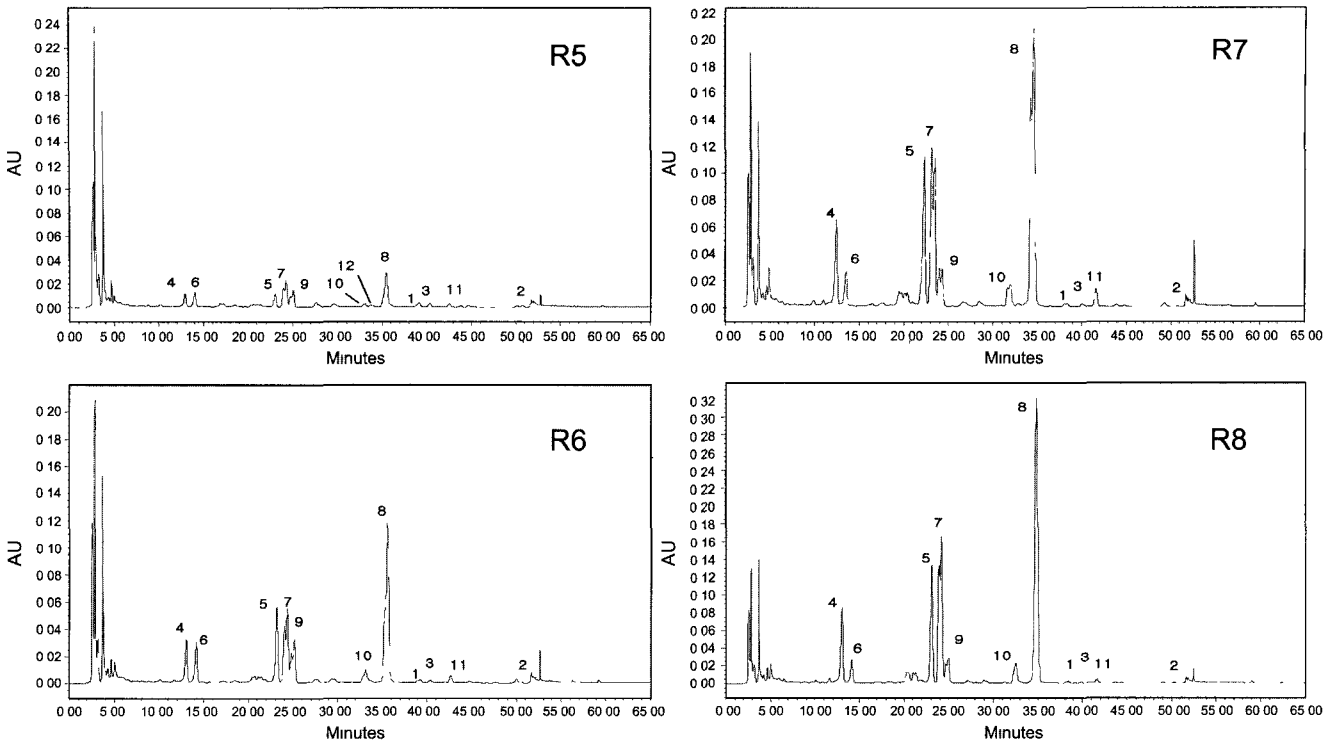

Fig. 3. Profiles of free isoflavone changes in Korean soybean variety, 'Sinpaldalkong 2' during the reproductive stages R5, R6, R7, and R8 stages. *1, daidzein, 2, genistein, 3, glycitein, 4; daidzin, 5, genistin, 6, glycitin, 7, malonyldaidzin, 8, malonylgenistin, 9, malonylglycitin, 10, acetyldadzin, 11, acetylgenistin, 12, acetylglycitin*

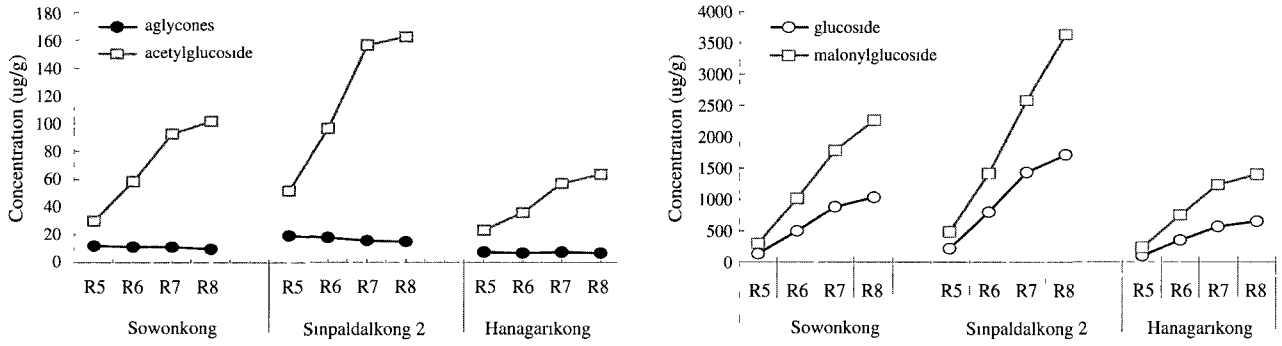

Fig. 4. Isoflavone contents in Korean soybean cultivars, 'Sowonkong', 'Sinpaldalkong 2', and 'Hanagarikong' during the reproductive stages R5, R6, R7, and R8 stages.

The 12 kinds of isoflavone were quantified by reverse-phased HPLC equipped with YMC-Pack ODS-AM303 column (4.6 × 250 mm) and the total isoflavone content was determined.

This experiment illustrates that the content and composition of isoflavone varied greatly among soybean cultivars, immature and mature soybeans of the same variety. The composition of isoflavone differed in each growth stage of soybeans. As seed maturity days progresses, total contents of isoflavone was increased in all cultivars (Table 1).

As seed maturity days progresses, isoflavone contents were increased until R8 stage, and the contents were high in the order of 'Sinpaldalkong 2' (5524.8 μg/g) > 'Sowonkong' (3408.7 μg/g) > 'Hanagarikong' (2134.8 μg/g) (Table 1). As shown in Fig. 3 & 4, it was noted that the malonyl glucosides are predominant form among conjugated isoflavones followed by glucosides, acetyl glucosides, and aglycones. The approximate composition rates of conjugated isoflavones throughout the whole seed development stages were malonyl glucosides (64.2%), glucosides (30.7%), acetyl glucosides (4.1%) and aglycones (0.9%), respectively.

Malnoylgenistin, malnoyldaidzin, genistin and daidzein

contents showed a notable accumulation throughout the whole seed development period, whereas acetyldaidzin showed only a little increase. However, the contents of isoflavone aglycones (daidzein, glycitein, and genistein), glycitin, malonylglycitin, acetylgenistin, and acetylglycitin not only showed a little changes but also decreased during the late stages of seed development (Table 1).

In general, genetic and environmental factors are known to affect the isoflavone content of the seed. Recent work suggests that although seeds are the principal site of isoflavone synthesis, some accumulation is due to transport from other plant organs (Dhaubhadel *et al.*, 2004). Jung *et al.* (2004) and Subramanian *et al.* (2004) reported that isoflavone synthase has been shown to be expressed only in embryos and seed coats, and not in the developing cotyledons, suggesting the majority of the isoflavones in the cotyledons are transported from other tissues. From this study results, it was suggested that the mechanisms underlying the effects of seed development characteristics on isoflavone accumulation require further investigation.

Mono- and oligo-saccharides

The mono- and oligo-saccharides found in the developing seeds of 'Sowonkong', 'Sinpaldalkong 2', and 'Hanagarikong' were fructose, glucose, maltose, sucrose, raffinose, stachyose, DP3, DP6, and DP7 (Fig. 5).

Sucrose and stachyose were presented as a major saccharide in soybean seeds. As maturing days progressed, they were constantly increased and the highest contents were observed at R8 stage. While small quantities of raffinose, fructose, glucose, maltose, DP3, DP6, and DP7 were detected by the HPLC system

Karr-Lilienthal *et al* (2005) reported that soybean carbohydrates make up approximately 35% of soybean seed dry matter, and the half of these carbohydrates are nonstructural in nature, including low molecular weight sugars, oligosaccharides, and small amounts of starch, while the other half are structural polysaccharides.

In this study, we found that the free glucose, fructose, maltose, and sucrose make up the low molecular weight sugars. Among the low molecular weight sugars, the contents of fructose, glucose, and maltose were constantly decreased during seed maturity. The oligosaccharides such as raffinose, stachyose, DP3, DP6 and DP7 in the developing seeds comprise approximately 1.31 ~ 3.95% (R5~R8) of the soybean dry matter. The primary oligosaccharide found in soybean seed was galacto-oligosaccharide such as raffinose and stachyose. Whereas, the little amount of DP3, DP6, and DP7 were detected in the developing soybean seeds, but they were gradually decreased and not detected at R6 ~ R7 stages.

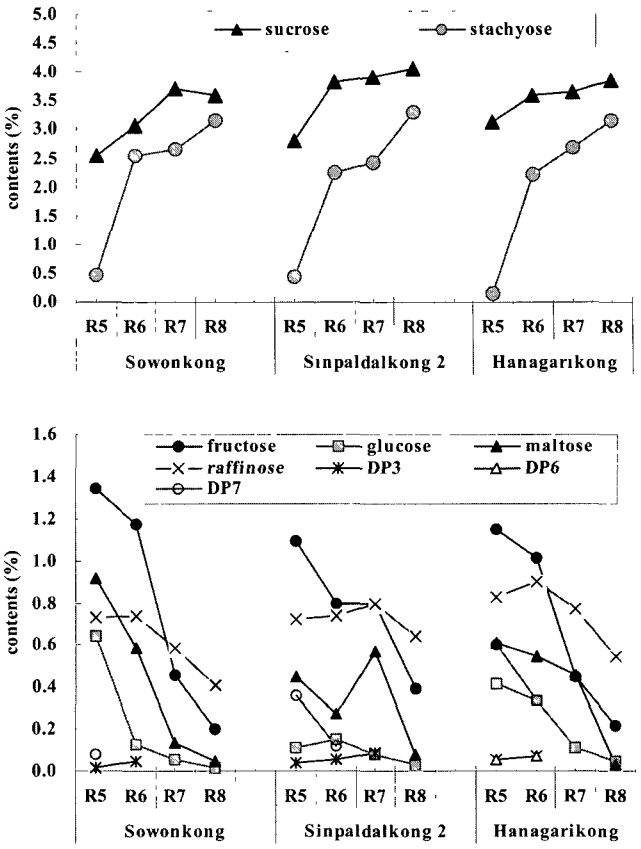

Fig. 5. Changes of mono- and oligo-saccharides contents in Korean soybean cultivars, 'Sowonkong', 'Sinpaldalkong 2', and 'Hanagarikong' during the reproductive stages R5, R6, R7, and R8 stages

It was reported that raffinose and stachyose begin to accumulate prior to physiological maturity, and the accumulation of raffinose oligosaccharides and galactosyl cyclitols have been characterized during soybean seed development (Yazdi-Samadi *et al.*, 1977; Amuti & Pollard, 1977; Dornbos & McDonald, 1986).

In this study results showed that saccharide composition at

Table 2. Relationship among isoflavone and mono-, oligo-saccharide contents in maturing soybean seeds

	Fruc	Gluc	Sucr	Malt	Raffi	DP3	Stach	DP6	DP7	Total
Isoflavone	-0.651 *	-0.653 *	0.780 **	-0.602 *	-0.372 ns	-0.178 ns	0.763 **	-0.339 ns	-0.586 *	0.575 *

*, ***significant at p<0.05, p<0.01 levels, respectively, ns not significant*

the beginning of seed development is primarily monosaccharides with little sucrose and oligosaccharides, but as seed development proceeds the sucrose and starch increase with concomitant decrease in the monosaccharides.

Sugars are important in all organisms, both as carriers of stored chemical energy and as raw materials for the synthesis of other molecules. Therefore, sugar concentrations affect the expression of a large number of genes and other chemical compounds (Koch, 1996).

As shown in Table 2, statistical analysis of correlation among mono- and oligo-saccharides with isoflavones of maturing soybean was conducted to determine whether these components affected the relative contents of isoflavones.

Obtained results show that sucrose and stachyose have a positive correlation with isoflavone ($r=0.780$, 0.764 at $p<0.01$, respectively), while fructose, glucose, maltose, and DP7 have a negative correlation with isoflavone ($r=-0.651$, -0.653, -0.602, and -0.586 at $p<0.05$, respectively). However, raffinose, DP3, and DP6 were not significantly correlated with isoflavone, but they showed the negative relationship.

Protein, amino acid, and free amino acid

Protein and amino acid accumulation were very rapid during reproductive stages, and reached the highest contents at R7 stage of 'Sinpaldalkong 2' and 'Hanagarikong', corresponding to maximum fresh weight/seed, while the contents of 'Sowonkong' were increased until R8 stage (Fig. 6).

The seventeen amino acids detected and most of amino acids increased until R7 stage, although there is some variation among the three cultivars, and then slightly decreased at R8 stage. Sulfur containing amino acids such as cystein and methionine increased until R8 stages in all three cultivars, whereas tyrosine and phenylalanine linearly decrease at the later stage of maturity (data not shown). These amino acid contents increased during reproductive stages but their percentage values remained relatively unchanged until R8 stage, and these results were supported by Yazdi-Samadi *et al* (1977).

The thirty four free amino acid detected and their contents sharply decreased throughout the reproductive stages, but the decreasing pattern of 'Sowonkong' were slightly different from other cultivars (Fig. 6). However, it needed a more detailed investigation because 'Sowonkong' is a variety mainly developed for soybean sprouts, and this fact also represents the possibility of differences as compared to other soybean cultivars.

Among the thirty four free amino acids, the contents of most acids were below than 5%, but five amino acids such as γ-amino-n-butyric acid, arginine, urea, glutamic acid, alanine showed approximately 57.8% of free amino acids, and their composition rate were 17.3%, 14.5%, 11.4%, 7.8%, and 6.8%, respectively. However, γ-amino-n-butyric acid decreased throughout the reproductive stages, while urea and arginine were linearly decrease at the later stage of maturity (*data not shown*).

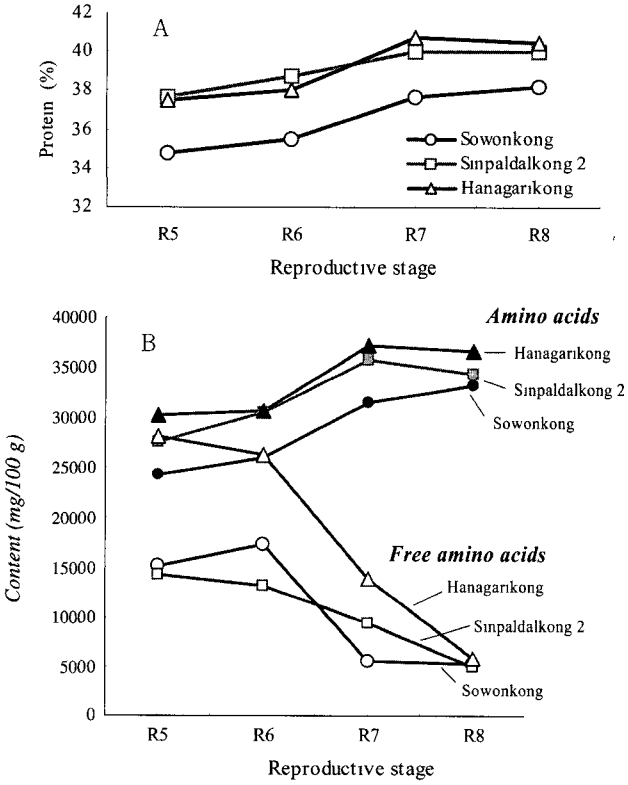

Fig. 6. Changes of protein (A), amino acid, and free amino acid contents (B) in Korean soybean cultivars, 'Sowonkong', 'Sinpaldalkong 2', and 'Hanagarikong' during the reproductive stages R5, R6, R7, and R8 stages. †*Amino acids represent the sum of cysteine, histidine, isoleucine, lysine, arginine, phenylalanine, proline, serine, valine, alanine, aspartic acid, glutamic acid, glycine, leucine, methionie, threonine, tyrosine* ‡*Free amino acids representes the sum of alanine, anserine, arginine, aspartic acid, α-amino adipic acid, α-amino-n-butyric acid, β-alanine, β-amino isobutyric acid, γ-amino-n-butyric acid, carnosine, citrulline, cystathionine, ethanol amine, glutamic acid, glycine, histidine, hydroproline, hydroxylysine, isoleucine, leucine, lysine, methionine, 1-methylhistidine, NH_3, ornithine, phenylalanine, phosphoserine, proline, sarcosine, serine, taurine, threonine, tyrosine, urea, valine*

Table 3. Relationship among isoflavone and protein, amino acid and free amino acid in the maturing soybean seeds.

	Protein	Amino acid	Free amino acid
Isoflavone	0.571*	0.599*	-0.673**

*, **: *significant at $p<0.05$, $p<0.01$ levels, respectively*

In general, young soybeans undergo many changes before reaching maturity and a major feature of these changes is mass syntheses of amino acids and storage proteins, and as a result there is an overall increase in dry matter.

These study results showed that Soybeans at R8 stage are high in protein and amino acid, but low in free amino acid contents and this results were supported by Minamide & Hata (1990).

We have reported in our previous study that the composition rates of 11S related proteins have a tendency to increasing whereas those of 7S related proteins have a tendency to decreasing during the maturing (Kim *et al.*, 2004).

On the basis of obtained results, we tried to understand the relationship with these nutrients and isoflavone contents in the maturing soybean seeds.

As shown in Table 3, protein (r=0.571) and amino acid contents (r=0.599) showed significant positive correlations ($p<0.05$) with isoflavone contents, but the free amino acid contents showed negative correlation with isoflavone (r= -0.673, $p<0.01$). It was reported that soybean isoflavones have antioxidant properties, which may protect LDL from oxidation (Wiseman *et al.*, 2000), and also soybean protein may exert its anti-atherogenic effects *via* associated isoflavones (St Clair, 1998; Vitolins *et al.*, 2001).

It was suggested that the accurate characterization of protein related chemical components and their relation to isoflavone synthesis in the developing soybean seed is necessary for the improvement of soybean utilization.

Lipid and fatty acids

The lipid content showed a relatively moderate increase. The highest content reaches at R7 stage in 'Sowonkong' and 'Sinpaldalkong 2', and at R8 stage in 'Hanagarikong', respectively (Fig. 7).

In this study result shows that the lipid content reaches its final content relatively early stage of seed development and remains constant as compared with other chemical components.

The fatty acid composition of maturing soybean seeds indicates that soybean seeds are abundant in unsaturated fatty acids, and lower amounts present in saturated fatty acids (Table 4). The composition rates of unsaturated fatty acids, such as oleic acid (18:1), linoleicacid (18:2), and linolenic acid (18:3) ranged approximately 79.7% (R8 stage of 'Sowonkong')~83.1% (R6 stage of 'Sinpaldalkong 2'). Meanwhile, major saturated fatty acid of maturing seeds is palmitic acid (16:0) which ranged 12.2% (R7, 'Sinpaldalkong 2')~14.6% (R8, 'Sowonkong') of total fatty acids. However, only small quantities of stearic acid (18:0) were observed.

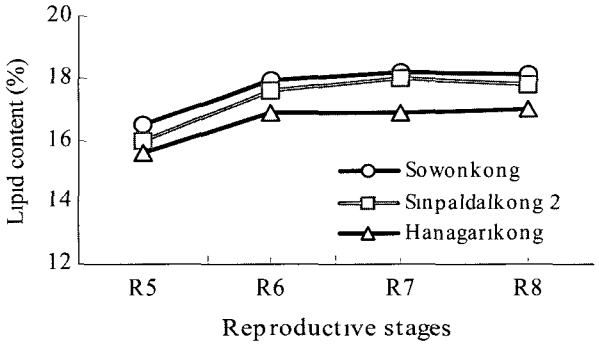

Fig. 7. Changes of lipid contents in Korean soybean cultivars, 'Sowonkong', 'Sinpaldalkong 2', and 'Hanagarikong' during the reproductive stages R5, R6, R7, and R8 stages.

Table 4. Changes of fatty acid composition in Korean soybean cultivars, 'Sowonkong', 'Sinpaldalkong 2', and 'Hanagarikong' during the reproductive stages R5, R6, R7, and R8 stages (Unit : %)

cultivars	RS§	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	SFA†	USFA‡
Sowonkong	R5	13.5±1.5	5.0±0.2	20.9±2.0	49.9±2.3	10.8±0.4	18.5±1.6	81.6±4.5
	R6	13.0±1.3	5.2±0.2	24.0±1.9	48.5±1.9	9.4±0.5	18.2±1.5	81.9±4.3
	R7	13.7±1.4	5.6±0.2	21.6±1.9	50.7±2.0	8.4±0.5	19.3±1.5	80.7±4.2
	R8	14.6±0.9	5.8±0.1	24.4±2.1	47.6±2.1	7.7±0.4	20.4±0.9	79.7±4.4.
Sinpaldalkong 2	R5	13.7±1.1	4.3±0.1	26.4±1.6	45.7±1.8	9.9±0.5	18.0±1.3	82.0±3.8
	R6	12.2±1.2	4.7±0.1	21.4±1.4	52.9±1.9	8.8±0.3	16.9±1.3	83.1±3.7
	R7	13.0±0.8	4.9±0.1	21.0±1.7	52.2±1.8	8.9±0.6	17.9±1.0	82.1±4.0
	R8	12.7±0.7	5.5±0.2	20.8±1.7	52.4±2.3	8.6±0.3	18.2±1.1	81.8±4.1
Hanagarikong	R5	14.5±1.0	3.5±0.2	22.3±2.1	50.1±1.7	9.7±0.2	18.0±1.2	82.1±3.8
	R6	12.9±1.1	5.0±0.2	22.1±2.2	51.6±1.8	8.4±0.6	17.9±1.2	82.1±4.6
	R7	12.8±0.9	5.0±0.1	19.9±1.9	54.0±2.3	8.3±0.3	17.8±1.1	82.2±4.5
	R8	12.6±1.1	4.6±0.1	17.5±0.9	57.3±1.2	7.9±0.3	17.2±1.2	82.7±2.4

§*RS Reproductive stage*, †*SFA Saturated fatty acid*, ‡*USFA Unsaturated fatty acid*

Table 5. Relationship among isoflavone and lipid, saturated fatty acid (SFA) and unsaturated fatty acid (USF) in the maturing soybean seeds.

	Lipid	SFA	USFA
Isoflavone	0.754**	0.193^{ns}	-0.221^{ns}

** : significant at p<0 01 levels, ns : not significant

Among the fatty acids, although varietal difference was presented, stearic acid and linolenic acid were gradually decreased, while oleic and linoleic acid were increased as seed maturing progressed.

As shown in Table 5, lipid and fatty acids in the maturing seeds were examined to ascertain whether these components affected the contents of isoflavones. Obtained results showed that lipid are significantly correlated ($r=0.754$, $p<0.01$) with isoflavones However, neither saturated fatty acid nor unsaturated fatty acids significantly affected the isoflavone contents of maturing soybean seeds.

ACKNOWLEDGEMENTS

This study was conducted by financial support of the BioGreen 21, the Rural Development Administration, Korea in 2003.

REFERENCES

Amuti, K. S. and C. J. Pollard 1977. Soluble carbohydrates of dry and developing seeds. Phytochemistry. 16 : 529-532.

Anderson, J. W., B. M. Johnstone, and M. E. Cook-Newell 1995 Meta-analysis of the effects of soy protein intake on serum lipids. New Engl. J. Med. 333 : 276-282.

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.

Dhaubhadel, S., B. McGarvey, R. Williams, and M. Gijzen. 2004. Isoflavanoid biosynthesis and accumulation in developing soybean seeds. Plant Mol. Biol. 53 : 733-743.

Dornbos, D. L. and M. B. McDonald. 1986. Mass and composition of developing soybean seeds at five reproductive growth stages. Crop Sci. 26 · 624-628.

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

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.

Hymowitz, T. and F. I. Collins. 1974. Variability of sugars content in seed of *Glycine max* (L.) Merrill and G *soja* Sieb. and Zucc. Agron. J. 66 : 239-240.

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.

Karr-Lilienthala, L. K., C. T. Kadzereb, C. M. Grieshopc, and G C. Fahey Jr.a, T. 2005. Chemical and nutritional properties of soybean carbohydrates as related to nonruminants· A review. Livestock Production Science. *In Press* Available online 2 April 2005.

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

Kim, S. L., H. T. Yun, J. K. Moon, K. Y. Park, Y. H. Lee, and Y. H. Ryu. 2004. Variations in seed storage protein among different colored soybean cultivars. Korean J. Crop Sci. 49(2) : 141-147.

Kinney, A. J. 1996. Development of genetically engineered soybean oils for food applications. J. Food Lipids. 3 . 273-292.

Koch, K. E. 1996. Carbohydrate-modulated gene expression in plants. Ann Rev. Plant Physiol. Plant Mol. Biol. 47 : 509-540

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

Kuwamura, S. and M. Tada. 1967. Isolation and determination of sugars from the cotyledon, hull, and hypeocotyl of soybean by carbon column chromatography. Tech. Bull. Fac. Agric Kagawa Univ. 18 : 138-141.

Lee, S. J. J. K. Ahn, S. K. Kim, J. T. Kim, S. J. Han, and I. M. Chung. 2003, Variation in isoflavone of soybean cultivars with location and storage duration. J. Agric. Food Chem. 2003, 51 : 3382-3389.

Minamide, T. and A. Hata. 1990. Effect of harvest time and storage temperature on the quality of green soybean seeds (*edamame*). Kyoto-furisu Daigaku Gakujutsu Hokoku, Seikatsu Kagaku, (Japanese) 41 : 23-28.

SAS Institute. 2000. SAS User's Guide Basics, 5th ed. SAS Institute, Cary, NC.

Schultz, W. B. 1998. Food labelling: health claims: soy protein and coronary heart disease. Fed. Reg. 63 : 62977-63015.

Spencer, M. M., D. Landau-Ellis, E. J. Meyer, and V. R. Pantalone. 2004. Molecular markers associated with linolenic acid content in soybean J. Am. Oil Chem. Soc. 81: 559-562.

St Clair, R. W 1998. Estrogens and atherosclerosis : phytoestrogens and selective estrogen receptor modulators. Curr. Opin. Lipidol. 9 : 457-463.

Subramanian, S., L. Xu, G Lu, J. Odell, and O. Yu. 2004. The promoters of isoflavone synthase genes respond differentially to nodulation and defense signals in transgenic soybean roots. Plant Mol. Biol. 54 : 623-639.

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 Amer-

can and Japanese soybeans in Iowa: Effects of variety, crop year, and location. J. Agric. Food. Chem. 42 : 1674-1677.

Wilson, R. F. T. C. Marquardt, W. P. Novitzky, J. W. Burton, J. R. Wilcox, and R. E. Dewey. 2001. Effect of alleles governing 16:0 concentration on glycerolipid composition in developing soybeans. J. Am. Oil Chem. Soc. 78 : 329-334.

Yazdi-Samadi, J., R. W. Rinne, and R. D. Sief. 1977. Components of developing soybeans seeds: Oil, protein, sugars, starch, organic acids, and amino acids. Agron. J. 69 : 481-486.

Vitolins, M. Z., M. S. Anthony, and G. L. Burke. 2001. Soy protein, isoflavones, lipids and arterial disease. Curr. Opin. Lipidol. 12, 433-437.

Wiseman, H., J. D. O'Reilly, H. Adlercreutz, I. M. Anthony, Elizabeth A. Bowey, I. R. Rowland, and A. B. Thomas. 2000.Isoflavone phytoestrogens consumed in soy decrease F2-isoprostane concentrations and increase resistance of low-density lipoprotein to oxidation in humans. Am. J. Clinical Nutrition. 72(2) : 395-400.