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Variations in Seed Storage Protein among Different Colored Soybean Varieties

Sun-Lim Kim†, Hong-Tae Yun, Jung-Kyung Moon, Keum-Yong Park, Yeong-Ho Lee, and Yong-Hwan Ryu

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

ABSTRACT: This study was carried out to know the variation of soybean seed proteins, 11S and 7S globulins, and their amino acid compositions among different colored soybean varieties, 'Danbaegkong' (yellow), 'Pureunkong' (green) 'Jinyulkong' (brown), and 'Geoumjeongkong 1' (black). Soybean seed proteins showed a wide range in molecular size, but the electrophoresis patterns of total seed protein subunits showed a similarity among different colored soybean varieties. Amino acid compositions of total seed proteins were similar for all soybean varieties tested. However, soybean varieties showed low composition rates in sulfur containing amino acids. The composition rates of cysteine and methionine in the 11S globulins were higher than those of total seed proteins and 7S globulins. Glutamic acid and glycine were higher in the 11S and 7S globulins than those of total seed proteins. However, the levels of methionine and phenylalanine are high in the 11S globulins, but those of valine and lysin are slightly lower than the 7S globulins. By using HPLC, we tried to analyse the soybean seed proteins. The 11S globulin was composed of 10 major peaks whereas the 7S globulin was composed of 4 major peaks. The composition rates of 11S related proteins have a tendency to increasing during the maturing whereas those of 7S related proteins have a tendency to decreasing. Composition rates of each peaks among different colored soybean varieties suggested that soybean seed proteins are varied, although they showed similarity in the electrophoresis patterns, and understanding of this characteristics is important for the utilization of soybeans.

Keywords: soybean, seed storage protein, 7S, 11S, amino acids

Soybean [*Glycine max* (L.) Merr.] has been utilized traditionally in many ways in Korea. Recently, breeders has been focused on developing new soybean varieties that have specific quality related traits such as high protein and high sulfur containing amino acids for protein sources, high isoflavone for health foods, no beany flavor for processing, black seed coat and green cotyledon for cooking with rice, high sugar content for vegetables.

The soybean seed coat determine the important quality traits, such as lustre, permeability, and nutritional value, and also seed coat color affects visual appearance of soyfoods. Soybean seed color showed it's diversity such as yellow seed coat and cotyledon, green seed coat and cotyledon, brown seed coat and yellow cotyledon, and black seed coat and yellow or green cotyledon. Soybean with black seed coat and yellow or green cotyledon which are called special soybeans usually being sold a premium price in the market for a cooking with rice, black soymilk, traditional medicine and other purposes.

Soybean is rich in protein, most of them are consisted of major seed storage proteins, 11S and 7S globulins, and accounted for 70% of the total seed proteins (Utsumi, 1992). The ratio of 11S/7S seed storage proteins can be used as an indicator of protein quality. The greater 11S/7S ratio implies the better nutritional quality of the seed protein (Peter et al., 1998).

The composition of subunits of 7S and 11S globulins varies in soybean varieties and legume species. It was reported that the gelation force of 7S globulin is mainly due to the hydrogen bond, whereas the gelation forces of 11S globulin are disulfide and hydrogen bond, and the disulfide bond of 7S globulin is limited because it contains only 2~3 cystine groups, but 11S globulin contains 6~37 sulfhydryl and disulfide groups per mole of protein (Kinsella 1979). The major 7S globulin exists as 6 isomers, each of which is composed of 3 discrete protein subunits, α', α and β-subunits, showing a molecular weight of 80, 76 and 50 kDa, respectively. The 11S globulin contains both acidic and basic subunits showing a molecular weight of 27~37 kDa (Thanh & Shibasaki, 1976). It was reported that gels prepared from 11S protein were more firm than those from 7S proteins, and the 11S content and 11S/7S protein ratio are correlated positively with tofu firmness (Delia & Wagner, 2002; Kang *et al.*, 1991; Saio *et al.*, 1969). However, other researchers found that the 7S globulin formed firmer tofu than the 11S globulin and there was no significant correlation between the 11S/7S ratio and tofu firmness (Taira, 1990). The 11S and 7S contents have been reported to be vary with soybean variety and environment (Cai & Chang, 1999; Saio *et al.*, 1969).

Seed proteins are classified into three major categories: storage proteins, structural proteins, and biologically active proteins. Of these proteins, storage proteins are the most abundant in seeds. Therefore, seed storage proteins have the greatest impact on the nutritional as well as processing prop-

†Corresponding author: (Phone) +82-31-290-6886 (E-mail) kimsl@rda.go.kr
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erties. Nutritional and functional qualities of protein are generally determined by its amino acid content and nitrogen solubility (Kinsella, 1979). This study was conducted to evaluate the constituents of soybean proteins in order to verify the variations of the 11S and 7S globulins, and amino acids among different colored soybean varieties.

MATERIALS ANDMETHODS

Soybean seeds

Four soybean varieties, 'Danbaegkong' (yellow), 'Pureunkong' (green) 'Jinyulkong' (brown), and 'Geoumjeongkong 1' (black), were grown and harvested at a field of National Institute of Crop Science, Suwon, Korea in 2002.

For the analysis of changing pattern of protein in soybean seeds during the reproductive stages, 'Sowonkong' seeds were sampled from the R5 through the R8 stages, and freeze dried and stored at 4 until analysis. Seeds were milled to flour using a cyclone mill and defatted with hexane using automatic fat extraction system (Gerhardt Soxtherm 2000, German).

Amino acid analysis

About 0.3 g of each sample was weighed and 5 m*l* 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 m*l* of Milli-Q water and filtered with Millipore 0.45 μm syringe filters (Milford, USA). The 1 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).

Extraction of total protein, 11S and 7S globulins

Defatted soybean flour were extracted with 0.03 M Tris-aminomethane buffer (pH 8.0) containing 0.01 M β-mercaptoethanol for 1 h with vortexing every 10 min. Then samples were centrifuged by 11,000 rpm for 20 min at room temperature, and the supernatant which contained the total seed proteins were collected and stored at 4 until analysis. The 11S and 7S globulins were extracted from defatted soybean flour according to the method of Thanh & Shibasaki (1976). Total seed protein extracts was adjusted to pH 6.4 with 2N HCl. The 11S globulin was collected by centrifugation, and analysed the extracts at pH 6.4 for 3 hr at 4°C. Crude 7S globulin was separated from the supernatant by adjusting to pH 4.8. The prepared 7S globulin was washed with pH 6.2 Tris-buffer and then dispersed into the standard buffer by adding 2 N NaOH to a pH 7.6 before adjusted back to pH 6.2. The solution was kept at 3~5 overnight. A trace of precipitate was finally removed by centrifugation. The obtained 11S and 7S globulins were then freeze dried and stored at 4 until analysis.

SDS-PAGE of total seed proteins, 11S and 7S globulins

Soybean proteins were analyzed using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), with the addition of β-mercaptoethanol as a reducing agent to protein. 50 mg of soybean flour was dispersed into 10 m*l* of 100 mM HEPES-sodium buffer (pH 8.0) and vortexed for 10s twice with a 5 min interval and then heated at 98°C in water bath for 5 min. The heated mixture was vortexed for 10s and then centrifuged for 20 min at 15,000 rpm. Immediately after centrifugation, the supernatant was applied to the Tris-glycine SDS-PAGE gels at a loading volume of 2 μ*l*. SDS-PAGE was performed using 4~20% Tris-glycine SDS-PAGE gradient gels (TEFCO Co., Japan). Electrophoresis was run at a constant voltage of 200 V in a vertical electrophoresis system (KOMA KE021, Korea). The running buffer (pH 8.4) was composed of 25 mM Tris, 190 mM glycine and 0.1% SDS. Gels were stained with See-band forte protein staining solution (Gene Bio-Application Ltd, Israel) for 1 h and destained two times with water/methanol/acetic acid solution (60/30/10, v/v) for 30 min. Protein MW marker II (TEFCO Co., Japan) was used as a protein molecular weight marker with bromchlorophenol blue as the tracking dye.

High performance liquid chromatography (HPLC) analysis

Total seed proteins extracted with 100 mM HEPES sodium buffer(pH 8.0) and the freeze-dried 11S and 7S globulins were dissolved in the buffer. Soybean seed proteins were separated with a reverse-phase C18 4.6×250 mm (5 μ) column (Phenomenex, USA) and Millenium^{32} HPLC workstation system (Waters, USA). The column temperature was 25°C, and the mobile phase consisted of 0.1% trifluoroacetic acid (TFA) in water (eluent A) and of 0.1% TFA in acetonitrile (eluent B). The flow rate was 1 min^{-1} and a 90 min gradient of 20~45% acetonitrile was followed by elution with 45% acetonitrile for 20 min, and spectra were monitored at 210 nm. The injection volume for all samples was 20 μ*l*.

RESULTS AND DISCUSSION

Electrophoresis profile of soybean seed proteins

SDS-PAGE profiles of soybean seed protein and their 11S

and 7S globulins are presented in Fig. 1. The crude soybean extracts contained many proteins covering a wide range of molecular masses, but the electrophoresis patterns of total seed protein subunits showed similarity among all soybean varieties which have different seed colors. However, the subunit of 'Danbaegkong' showed slightly lower density than other varieties (Fig. 1, SDS-PAGE I). The 7S globulin is composed of three subunits (α, α' and β) and all subunits are glycosylated and the α and α' subunits are processed at N-terminal regions. Glycinin is composed of acidic and basic polypeptides linked by a disulfide bond, but it is not glycosylated (Utsumi, 1992).

An amino acid sequence of each subunit is variable and glycinin exhibits polymorphism in the subunit composition among the cultivars (Maruyama *et al.*, 1998). In the electrophoresis patterns of 11S and 7S globulins from four soybean varieties, the polypeptide subunits of 11S and 7S proteins were well separated by SDS-PAGE. The 7S globulin was separated into subunits α and α', and the 11S globulin was separated into acidic and basic.

However, the qualitative profiles of the proteins of each of the globulins appeared to be similar among different colored soybean varieties (Fig. 1, SDS-PAGE II).

Momma *et al.* (1997) reported that among 11 varieties of soybean, three green and one black soybeans lacked a 26 kDa band which was found in all yellow soybeans. Lindstrom & Vodkin (1991) reported that dominant *I* gene inhibits its accumulation of anthocyanins in the soybean seed coat and the 35 kDa protein was abundant in the genotype *I/I* (yellow seed coat) whereas it was much reduced in the genotype *i/i* (imperfect black seed coats). In this study, however, we could not found any differences on the regions of 26 kDa and 35 kDa proteins of the different colored soybean seeds. Depending on this results, it was considered that the more detailed analysis at the molecular level of the electrophoresis should be performed for determining the exact differences of protein composition among the different colored soybeans.

Amino acid composition

Amino acid compositions of total seed proteins, 11S and 7S globulins are presented in Fig. 2. The amino acid composition of total seed proteins was similar for all soybean varieties, although they have different seed coat colors. The obtained results noted that glutamic acid is the most abundant amino acid in soybean proteins. The acidic amino acids such as glutamic and aspartic acid constitute approximately 25% of the total amino acids and the basic amino acids such as lysine, arginine and histidine constitute approximately 20% of the total amino acids. However, all soybean varieties showed low content in sulfur containing amino acids. The composition of cysteine and methionine in the 11S globulin is about 3.0~4.5%, but the 7S globulin is deficient in sulfur containing amino acids (Coates *et al.*, 1985; Peter *et al.*, 1998; Fukushima, 1991).

It is well known that soybeans are deficient in the sulfur containing amino acids, so the sulfur containing amino acids

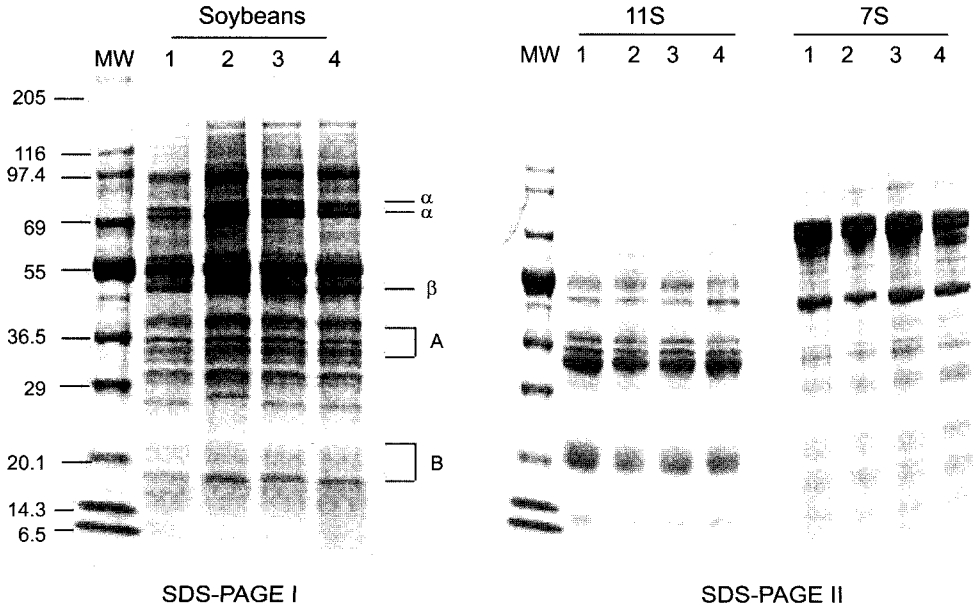

Fig. 1. SDS-PAGE patterns of total seed proteins (SDS-PAGE I), 11S and 7S proteins (SDS-PAGE II) from different colored soybean varieties. α', α, and β indicate subunits of β-conglycinin. A and B indicate acidic and basic polypeptides of glycinin, respectively. Lane MW: molecular weight marker; lane 1: 'Danbaegkong'; lane 2: 'Pureunkong'; lane 3: 'Jinyulkong'; lane 4: 'Geoumjeongkong 1'.

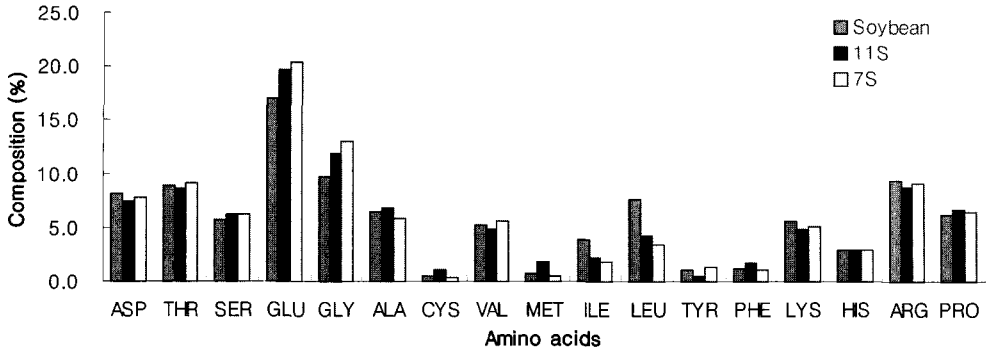

Fig. 2. Amino acid composition of total soybean protein, 11S and 7S proteins. All data represent the mean values of four different colored soybean varieties, 'Danbaegkong', 'Pureunkong', 'Jinyulkong', and 'Geoumjeongkong 1'.

are considered as the first limiting amino acid in nutrition. For this reason, there are now several breeding strategies including genetic engineering for increasing the sulfur containing amino acids in soybeans.

In this study, we attempted to isolate the 11S and 7S globulins from the total seed proteins, then analyzed their amino acids composition.

As compared the amino acid compositions of total seed proteins to the 11S and 7S globulins, the composition rates of cysteine and methionine for the 11S globulins were higher than those of total seed proteins and 7S globulins. Among amino acids, both glutamic acid and glycine were higher in the 11S and 7S globulins than those of total seed proteins (Fig. 2).

Marcone (1999) reported that 11S globulin contains higher levels of essential amino acids such as tryptophan, methionine, lysine, histidine, phenylalanine, valine and isoleucine than the 7S globulin. However, the obtained results of this study show the composition rates of methionine and phenylalanine are high in the 11S globulin, but those of valine and lysin are slightly lower than the 7S globulins. In addition, we observed the interesting results that the composition rates of glutamic acid and glycine are higher in the 7S globulins than those of total seed protein and 11S globulins.

HPLC analysis of total seed protein, 11S and 7S globulins

We tried to determine characteristics of the soybean seed proteins, and their 11S and 7S globulins by using the HPLC. As shown in Fig. 3, the weak hydrophobic polypeptides of the 11S and 7S were separated earlier than strong hydrophobic polypeptides, and the 11S globulin of 'Danbaegkong' was composed of the 10 major peaks(1~8 and 12), whereas the 7S globulin was composed of the 4 major peaks(9, 10, 11, and 14).

In general, soybean seeds begin to growth when soybeans are reached at R5 stage, and at this stage the seed sizes are

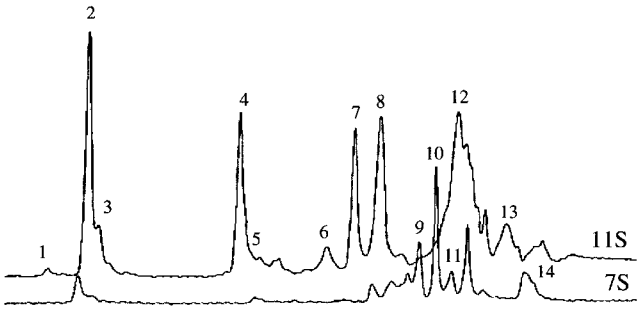

Fig. 3. HPLC chromatogram profiles of the 11S and 7S proteins separated from Korean soybean variety 'Danbaegkong'. Chromatogram are obtained by using a revers-phase Jupiter C18 4.6×250 mm (5 μ) column, 0.1% TFA in water (eluent A) and 0.1% TFA in acetonitrile (eluent B). Flow rate was 1 m*l* 1 min^{-1}, 90 min gradient of 20-45% acetonitrile, and monitoring at 210 nm.

approximately 3 mm long. At R6 stage soybean seeds are in the state of full seed-pod containing green seed that fill the pod cavity.

When soybean seed 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.

As shown in the Fig. 4, the content of each component base on peak area was changed according to the reproductive stages. Peak 12 which is considered as a components of the 11S globulin was the most abundant protein in 'Sowonkong', and it was ranged from 38.0~39.7% and slightly decreased as soybean seeds matured. Whereas, peak 9 and 10 which are considered as a components of the 7S globulin were ranged from 7.5~8.6% and 17.2~26.9%, respectively. As the reproductive stages were proceeded, they were much more deceased than the 11S proteins and, furthermore, peak 9 was disappeared after the R6 stage. From the obtained results, we could suggest that the composition rates of the 11S related proteins have a tendency to

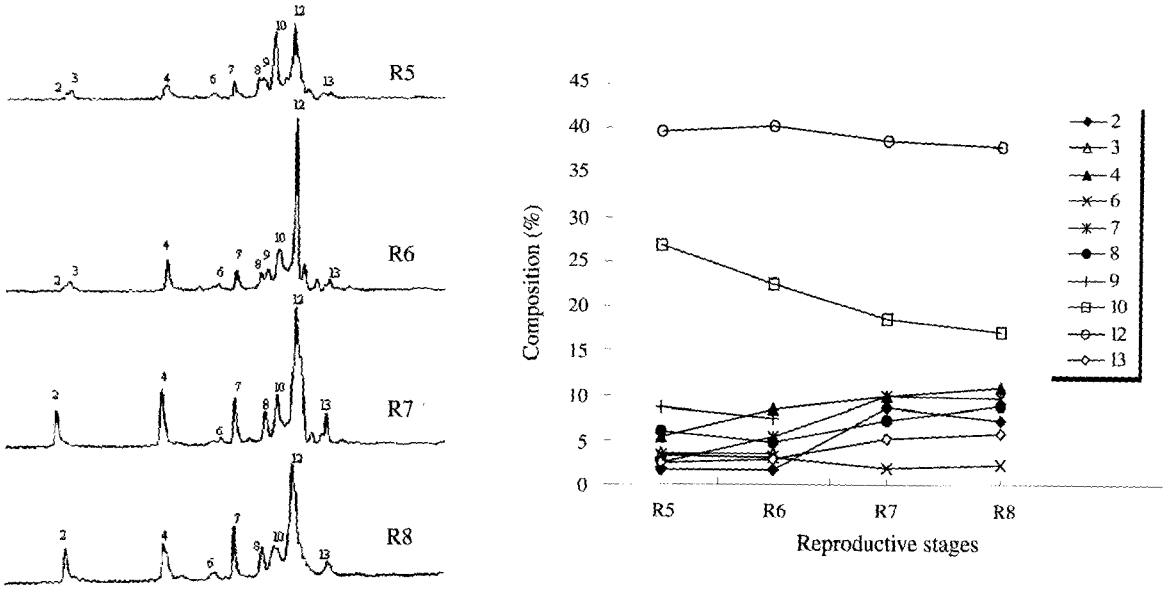

Fig. 4. Profiles of HPLC chromatogram and changes of composition rates of total seed proteins during the reproductive stages of Korean soybean variety 'Sowonkong'.

increase whereas the rates of 7S related proteins have a tend to decrease during the maturing. However, we also noted that 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 types of soybean varieties.

Comparisons were made to determine the differences among different colored soybean varieties. Among the tested soybean varieties, no apparent differences were found in the chromatogram of 'Danbaegkong', 'Pureunkong', and 'Jinyulkong'. Only the difference for the chromatogram of 'Danbaegkong', 'Pureunkong', and 'Jinyulkong' was the abundance of each peaks except the 11 peak of 'Pureunkong'. This results were similar with the results of Raijni *et al.* (2003) in which they could not found apparent differences in the HPLC chromatogram patterns of the 7 soybean varieties. However, the HPLC chromatogram pattern of 'Geoumjeongkong 1' which has black seed coat was more different than those of 'Danbaegkong', 'Pureunkong', and 'Jinyulkong' which have yellow, green, and brown seed coat, respectively. In the HPLC chromatogram of 'Geoumjeongkong 1', we noted that the peak 1, 5, and 6 which were considered as the components of the 11S protein, and peak 14 which was considered as the component of 7S protein were not detected in other soybean varieties. This results suggested that 'Geoumjeongkong 1', although they showed the similar SDS-PAGE pattern to other varieties (Fig. 1), have different protein pattern than other soybean varieties.

Composition rates of each peaks among different colored soybean varieties were calculated and presented in Table 1. As shown in the Table 1, the peak 12 was found as a most abundant protein and it was ranged from 25.8 to 38.9%. In 'Danbaegkong', three peaks such as peak 2, 7, and 12 were found as the major protein peaks and they comprised about 47.2% of the 14 protein peaks. In 'Jinyulkong', two peaks such as peak 12 and 10 were found as the major protein peaks and they were comprised about 48.5% of the 13 protein peaks. In 'Geoumjeongkong', three peaks such as peak 12, 13, and 7 were found as the major protein peaks and they were comprised about 63.3% of the 9 protein peaks. This results implied that soybean seed proteins are varied according to the seed colors, therefore, the understanding of this characteristics is very important for the utilization of soybeans.

Soybean seeds have a wide variety of phenotype including coloration, size, shape, luster, and permeability. In general, black colored soybeans are different from the commonly grown yellow colored types. This trait is determined by the *I* locus, a cluster of chalcone synthase genes that control anthocyanin biosynthesis in the seed coat (Todd & Vodkin, 1996). There is also variation in the proteins from seed coats of different soybean varieties, and corresponding genes encoding both structural and soluble seed coat proteins have been isolated (Gijzen *et al.*, 1993; Lindstrom & Vodkin, 1991).

In the domestic market of Korea, most of the colored soybeans are sold at a premium price and most of them are used for various purpose such as black or green colored tofu, Meju or Deonjang (fermented soy paste), Kongjaban

Table 1. Comparison of relative content of individual proteins among different colored soybean varieties.

Peaks	Soybean varieties			
	Danbaegkong (yellow)	Pureunkong (green)	Jinyulkong (brown)	Geoumjeongkong 1 (black)
1	2.7±0.2	1.1±0.1	1.0±0.1	–
2	10.4±1.1	8.6±0.8	10.4±1.2	3.3±0.3
3	5.3±0.6	4.9±0.4	3.5±0.5	0.6±0.0
4	8.1±0.9	7.1±0.5	8.6±1.1	–
5	2.1±0.2	1.1±0.2	0.8±0.0	–
6	1.8±0.1	1.1±0.1	1.8±0.3	–
7	11.0±1.1	8.3±0.7	8.7±0.6	13.9±1.5
8	8.0±0.4	6.8±0.6	3.9±0.2	7.8±1.0
9	5.1±0.2	3.6±0.2	2.5±0.2	8.6±0.8
10	3.9±0.2	11.6±0.6	1.7±0.0	5.9±0.2
11	3.0±0.1	–	3.7±0.1	4.1±0.4
12	25.8±2.6	36.9±3.1	36.7±2.7	38.9±2.9
13	9.6±0.8	7.4±1.0	16.2±1.3	16.9±1.2
14	3.1±0.1	1.5±0.1	0.5±0.0	–

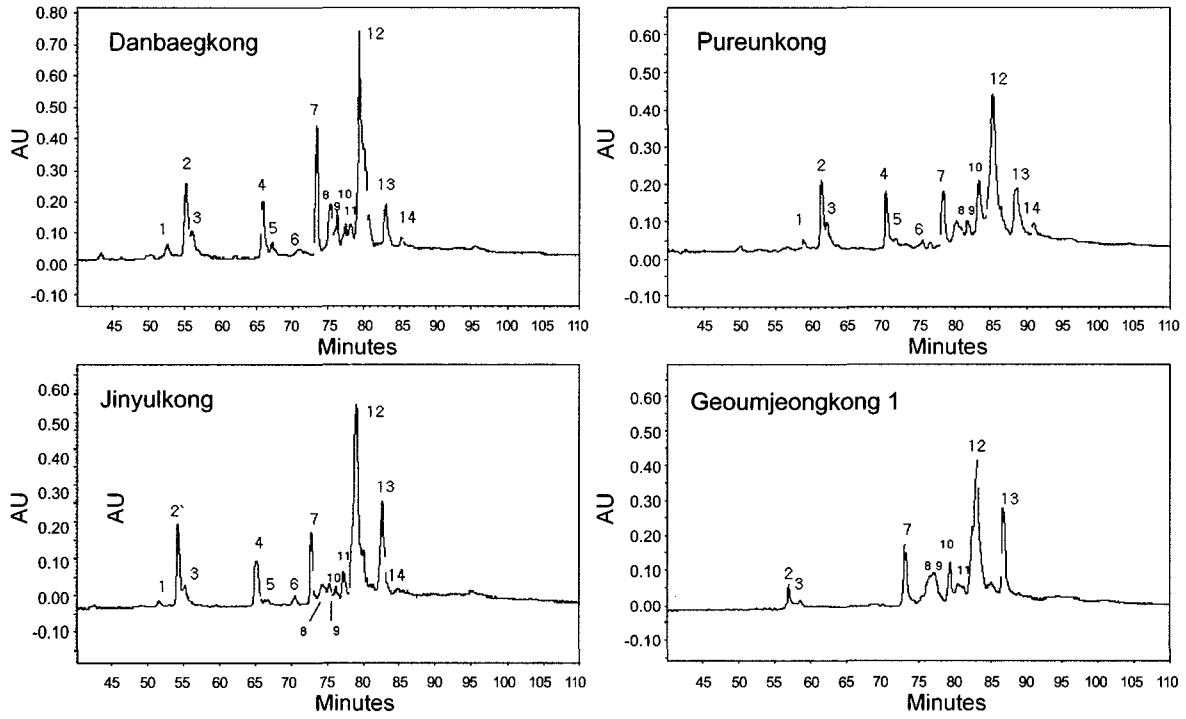

Fig. 5. Comparison of HPLC chromatogram of total seed proteins among different colored soybean varieties 'Danbaegkong', 'Pureunkong', 'Jinyulkong', and 'Geoumjeongkong 1'.

(cooked with soy sauce), cooking with rice, and soybean sprouts. The listed soybean foods are major plant protein sources for Koreans. In the point of plant protein source, however, the differences among different colored soybeans are not clear and no reports were made so far. So we think that it is a very important to determine the protein property of different colored soybeans for supporting the proper usage of special soybean varieties. This results indicate that protein composition, especially, the content of glycinin (11S) and β-conglycinin (7S), their ratios, and the level of specific 11S and 7S subunits are important in the application of soybean grain as foodstuff.

REFERENCES

Cai, T. and K. C. Chang. 1999. Processing effect on soybean storage proteins and their relationship with tofu quality. J. Agric. Food Chem. 47 : 720-727.

Coates, J. B., J. B. Mederiros, V. H. Thanh, and N. C. Nielsen. 1985. Characterization of the subunits of -conglycinin. Arch. Biochem. Biophys. 243 : 184-194.

Delia, A. S. and J. R. Wagner. 2002. Comparative study of foaming properties of whey and isolate soybean proteins. Food Res. Inter. 35 : 721-729.

Fukushima, D. 1991. Recent progress of soybean protein foods: Chemistry, technology, and nutrition. Food Rev. Int. 7 : 323-351.

Gijzen, M., R. van Huystee, and R. Buzzell. 1993. Soybean seed coat peroxidase: a comparison of high-activity and low-activity genotypes. Plant Physiol. 103 : 1061-1066.

Kang, L. J., Y. Matsumura, and T. Mori. 1991. Characterization of texture and mechanical properties of heat-induced soy protein gels. J. Am. Oil Che. Soc. 68 : 339-345.

Kinsella, J. E. 1979. Functional properties of soy proteins. J Am. Oil Chem. Soc. 56 : 242-258.

Landstrom, J. T. and L. O. Vodkin. 1991. A soybean cell wall protein is affected by seed color genotype. Plant Cell 3(6) : 561-571.

Marcone, M. F. 1999. Possible nutritional implications of varietal influence on the 7S/11S seed globulin ratios in Amaranth. Plant Foods for Human Nutrition 54(4) : 375-380.

Maruyama, N., T. Katsube, Y. Wada, M. H. Oh, A. P. Barba de la Rosa, E. Okuda, S. Nakagawa, and S. Utsumi. 1998. The roles of the N-linked glycans and extension regions of soybean -conglycinin in folding, assembly and structural features. Eur. J. Biochem. 258 : 854-862.

Momma, M., K. Haraguchi, M. Saito, K. Chikuni, and K. Harada. 1997. Purification and characterization of the acid soluble 26kDa polypeptide from soybean seeds. Biosci. Biotech, Biochem. 61 : 1286-1291.

Peter, J. S., N. C. Paek, and R. M. Shibles. 1998. Effects of nitrogen source and timing of sulfur deficiency on seed yield and expression of 11S and 7S seed storage proteins of soybean. Field Crop Res. 59 : 1-8.

Rajni, M., T. T. Dianne, and K. W. N. Perry. 2003. Characterization of storage proteins in different soybean varieties and their relationship to tofu yield and texture. Food Chemistry. 82(2) : 265-273.

Saio, K., M. Kamiya, and T. Watanabe. 1969. Food processing characteristics of soybean 11S and 7S proteins. Part I. Effect of difference of protein components among soybean varieties on formation of tofu gel. Agric. Biol. Chem. 33 : 1301-1308.

Taira, H. 1990. Quality of soybeans for processed foods in Japan. J. Agric. Res. Quality 24 : 224-230.

Thanh, V. H. and K. Shibasaki. 1976. Major proteins of soybean seeds. A straightforward fraction and their characterization. J. Agric. Food Chem. 24 : 1117-1121.

Thanh, V. H., K. Okubo, and K. Shibasaki. 1975. Isolation and characterization of the multiple 7S globulins of soybean proteins. Plant Physiol. 56 : 19-22.

Todd, J. J. and L. O. Vodkin. 1996. Duplications that suppress and deletions that restore expression from a chalcone synthase multigene family. Plant Cell 8 : 687-699.

Utsumi, S. 1992. Plant food protein engineering. In: Kinsella J. E., Editor, Advances in Food and Nutrition Research, Vol. 36, Academic Press, San Diego, CA : pp. 89-208.