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Changes of Phenol Compounds according to Storing Years in Soybean

Seung Hyun Kim‡, Hong Keun Song‡, Joung Kuk Ahn, Jung Tae Kim, Joon Sang Hahn, and Ill Min Chung†

Department of Crop Science, College of Life and Environmental Sciece, Konkuk Univ., Seoul, South Korea, 143-701

ABSTRACT: The objective of this study was to determine the role of storing years with the variation of total phenol and individual phenolic compounds in soybean (*Glycine max* L.) seeds. The total phenol content varied from 0.36 to 0.42% over four years, with the highest value (0.42%) found at storage for two and three years. Among the nine soybean varieties examined, Daweonkong had the highest total average phenol content (0.58%). The total content of 11 phenolics varied from 730.0 to 1812.8 μg g^{-1} over storage for four years, and the highest concentration (1812.8 μg g^{-1}) was found at storage for two years. Myeongjunamulkong (1465.4 μg g^{-1}) had the highest mean content among the nine soybean varieties. The total content of 11 phenolic compounds measured in this study occupied from 20.96 to 47.73% of the total phenol contents. The highest total phenol contents were in seeds with black coats (5279.4 μg g^{-1}), while the highest concentration of individual phenolic compounds were in seeds with green coats (1419.5 μg g^{-1}). Our study suggests that it may be feasible to improve soybean varieties with high functional substances such as phenolic compounds.

Keywords: soybean, storing year, total phenol contents, phenolic compounds, HPLC

The soybean (*Glycine max* L.) originates from northeast Asian regions such as China and Korea, where they have long been cultivated as a popular summer crop. In Korea, about 105,089 M/T were produced in 2003 (NAQS). Soybean seeds& contain phenol compounds such as chlorogenic acid, caffeic acid, ferulic acid & *p*-coumaric acid, which induce characters such as bitterness and astringency (Okubo *et al.*, 1992). They are also related in terms of antioxidation effects (Kim *et al.*, 1995, Chung *et al.*, 2000). Phenol compounds are classified into four groups: phenolic acid (C_6-C_1), coumarins (C_6-C_3), flavonoids (C_6-C_3-C_6) and condensed tannins (Lee & Lee, 1994). Mega & Lorenz (1974) noted that syringic, ferulic and vanillic acid were the main components in soybean seeds, while Dabrowski & Sosulski (1984) largely isolated syringic, *t*-ferulic, *p*-hydroxybenzoic, *P*-coumaric and *t*-caffeic acids. In particular, chlorogenic acid and caffeic acid, and hydrolytic compounds of both of these, have very high antioxidative effects (Hayes *et al.*, 1997). Lee & Lee (1994) reported that phenolic compounds had high antioxidant potentials under the lipid-aqueous system.

Generally, the analysis of individual phenolic compounds is undertaken using high performance liquid chromatography (HPLC) and other chromatography methods such as GC and TLC (Banwart *et al.*, 1985, Wieslaw *et al.*, 1988, Stanislaw *et al.*, 1990). The analysis of total phenol contents is widely conducted using spectrophotometric methods such as Folin-Dennis, Prussian blue and vanillin-HCl (Mega & Lorenz, 1994). Analysis of individual phenolic compounds using HPLC and total phenol contents using the spectrophotometer in soybean seeds also give similar results and are both up-to-date methods. In general, individual phenolic compounds among the total phenol contents in soybean seeds occupied from 28 to 72% (Seo & Morr, 1984). Lee & Lee (1994) reported that there were from 0.23 to 0.32% total phenol contents in soybean seeds using the Folin-Dennis method, measured from an equivalent curve using tannic acid. Seo & Morr (1984) detected total phenol contents of 4 mg g^{-1} in defatted soybean flakes and Mega & Lorenz (1974) reported that individual phenolic acid contents in defatted soybean seeds were 256 μg g^{-1} using HPLC. Pratt *et al.* (1982) and Dabrowski & Sosulski (1984) reported that the following compounds were found: chlorogenic acid (2.8×10^{-2} moles kg^{-1}), caffeic acid (1.1×10^{-4} moles kg^{-1}), *p*-hydroxybenzoic acid (139 μg g^{-1}), *p*-coumaric acid (94 μg g^{-1}), and *t*-caffeic acid (60 μg g^{-1}).

There is very little information available on the chemical analysis of soybean seeds stored for several years. Therefore, we decided to study the change of the phenol composition and concentrations of soybean seeds at room temperature over such a time-period.

The main purpose of this study was to investigate the change of total phenol content and individual phenolic compounds over several storing years. Such basic information may suggest the improvement of the storing environment and chemical substances present in soybean seed for use as natural functional foods.

†Corresponding author: (Phone) +82-2-450-3730 (E-mail) imcim@konkuk.ac.kr <Received March 22, 2004>

‡These two authors contributed equally to this work

MATERIALS AND METHODS

The nine soybean varieties, including Muhankong, used for this experiment were cultivated at Suwon in 1998, 1999, 2000 and 2001. Table 1 showed climatic data in cultivatied time. The varieties differed in maturity, height, seed weight, growing habit and other characteristics. The soil was a silt clay loam at all sites and the previous crop was soybean. For this reason, no artificial inoculation was needed. The planting arrangement was 60×15 cm per plot and the plants were thinned to a uniform density 14 days after planting. Appropriate pesticides were used to control weeds, diseases and insects, and fertilizers were applied prior to plowing at the recommended rates of 8, 8 and 12 kg per 1000 m^2 for N, P_2O_5 and K_2O, respectively. Each plot consisted of three rows (3.75 m long and 0.6 m between rows) and the experiment consisted of a completely randomized design with three replicates. Soybean seeds were harvested from each replicate for each cultivar at each crop year (1998, 1999, 2000 and 2001) and stored at room temperature for analysis of total phenol and 11 individual phenolic compounds in 2002.

Quantitative analysis of total phenol

Soybean seeds with their seed coats were ground and 2 g of the powder extracted with 80% methyl alcohol for 1 day in a shaker-chamber at 25°C with stirring. The extracts were filtered through a Whatman No. 42 filter paper and the quantitative analysis of total phenols was measured by the Folin-Dennis method (A.O.A.C, 1980). Half a ml of the extract sample, 5 ml of distilled water and 5 ml of the Folin and Ciocalteau phenol reagent were mixed in a screw-top flask. After 3 min, 2 ml of 10% sodium carbonate (Na_2CO_3) was added and the mixed solution stirred in a lit shaker-chamber at 30°C for 1 h. After the reaction, the absorbance of the solution was measured with a spectrophotometer (Hitachi Ltd., Tokyo, Japan) at 760 nm. The standard curve was measured with 1, 50 and 100 ppm of ferulic acid purchased from Sigma Aldrich (St. Louis, U.S.A.).

Extraction conditions of the soybean seeds

Each 2 g sample of whole soybean seed was ground, mixed with 2 ml of 0.1 N HCl and 10 ml of acetonitrile (ACN) in a 125 ml screw-top flask and stirred for 2 h at room temperature. After the samples were stirred, the solution was filtered through a Whatman No. 42 filter paper. The filtrate was dried under vacuum at a temperature below 30°C and then redissolved in 10 ml of 80% HPLC grade methyl alcohol in distilled water. The redissolved sample was filtered through a 0.45 μ filter (Cameo 13N syringe-filter, nylon) and transferred to a 1 ml vial.

Quantitative analysis of 11 individual phenolics using HPLC

The HPLC system consisted of a Young-Lin M930 liquid chromatograph pump and a M720 detector (Young-Lin Instruments CO., LTD, Korea). The reversed-phase column for quantitative analysis was an YMC-Pack ODS-AM-303 (250×4.6 mm I.D.) and the UV absorption was measured at 280 nm. The mobile phase consisted of solvents A and B. Solvent A contained 98% water and 2% glacial acetic acid in 0.018 M ammonium acetate. Solvent B was 70% solvent A and 30% organic solvent. The organic solvent contained 82% methanol, 16% *n*-butanol, and 2% glacial acetic acid in 0.018 M ammonium acetate. The following gradient was used: 0.0 to 1.0 min, isocratic at 10% B; 1.0 to 21.0 min, linear gradient from 10 to 25% B; 21.0 to 36.0 min, linear gradient from 25 to 45% B; 36.0 to 56.0 min, linear gradient from 45 to 100% B; 50.00 to 50.15 min, flow increased to 1.20 ml min^{-1}; 82.00 to 82.15 min, linear gradient from 100 to 10% B; 92.00 to 92.15 min, flow decreased to 1.00 ml min^{-1}, and at 99.0 min the sampled loop was rinsed and the gradient repeated (Banwart *et al.*, 1985).

Calibration curves of eleven individual phenolics

The 11 individual phenolic standards, including ferulic acid, were purchased from Sigma Aldrich (U.S.A) in 2001 and

Table 1. Climatic information at Suwon in cropping years.

	1998 year			1999 year			2000 year			2001 year		
	July	Aug.†	Sept.‡	July	Aug	Sept	July	Aug	Sept	July	Aug	Sept
Temperature (°C)	25.2	25.6	23.0	25.5	25.5	22.9	26.9	25.9	20.1	25.5	25.7	21.6
Sunshine duration (hr)	151.0	134.0	190.3	164.6	192.2	156.1	161.8	151.8	150.9	132.8	223.3	223.8
Precipitation (mm)	306	591.6	141.2	345.0	338.4	402.2	375.8	448.8	182.2	469.7	144.7	12.1
Relative humidity (%)	74.4	76.1	76.5	77.8	77.8	81.2	75.2	76.8	71.8	77.2	69.6	59.4
Average wind velocity (m sec^{-1})	2.6	1.9	1.7	2.2	1.9	1.6	2.1	2.1	2.0	2.0	2.1	2.3

†August, ‡September

used for calibration curves. The purity of each standard was determined by HPLC chromatography, and then obtained by plotting standard concentration at five concentration, 1 ppm, 25 ppm, 50 ppm, 75 ppm and 100 ppm, respectively. High linearity (r^2>0.99) was obtained for each curve. Ferulic, gentisic, chlorogenic, caffeic, *p*-coumaric, *p*-hydroxybenzoic, salicylic and syringic acids, as well as hesperidin, narigin, and hyricetin were identified by their retention times or by co-chromatography with authentic standards. Concentrations were calculated by comparing peak areas of samples with those of the standards.

Statistical analysis

Analysis of variance for all data was undertaken using the general linear model (GLM) procedure of the statistical analysis system (SAS, SAS Institute, Inc., 2000). All of the aforementioned experiments were replicated three times using a completely randomized design. The pooled mean values were separated based on least significant difference (LSD) at the 0.05 probability level.

RESULTS AND DISCUSSION

Variation of total phenols in the nine soybean varieties over several storing years

The analysis of total phenol was conducted using the Folin-Dennis method and the calibration curve was made from ferulic acid and using the equation: $y=0.0011x+0.0612$ (r^2=0.999). The total phenol contents varied from 0.26 to 0.80% (dried soybean powder). Total phenol contents (0.44%) were the highest at storage for two and three years, lowest (0.36%) at storage for four years and the difference between the two variables was significant (Fig. 1). With regard to soybean varieties, Daweonkong had the highest mean total phenol content (5789.8 μg g^{-1}), Muhankong had

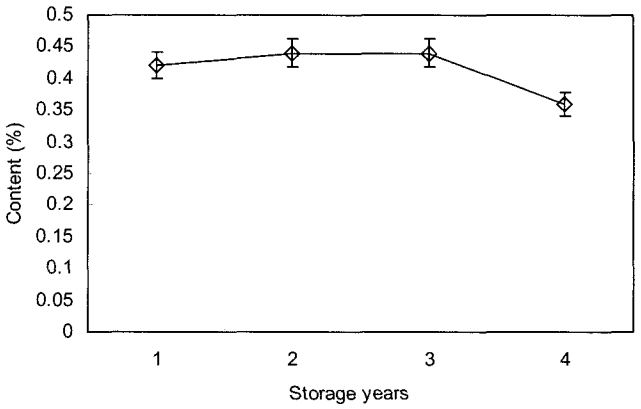

Fig. 1. The variation of total phenol contents in soybean seeds stored for four years (Vertical bars are represented of the standard error values).

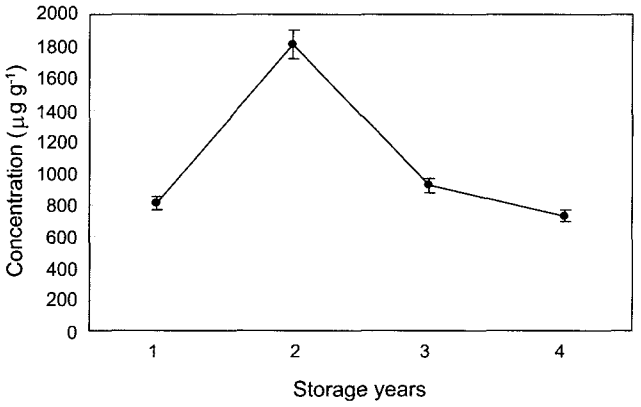

Fig. 2. The variation of individual phenolic compounds in soybean seeds stored for four years (Vertical bars are represented of the standard error values).

Table 2. The variation of total phenol contents among the nine soybean varieties stored for four years.

Variety \ Storing years	1	2	3	4	CV (%)	LSD (0.05)
	Ferulic acid equivalent (%)					
Muhankong	0.28	0.26	0.37	0.36	19.5	0.12
Daweonkong	0.60	0.80	0.48	0.44	12.1	0.13
Myeongjunamulkong	0.44	0.31	0.31	0.35	23.5	0.16
Jinpumkong 2	0.35	0.39	0.40	0.32	20.3	0.14
Taekwangkong	0.34	0.34	0.38	0.33	11.5	0.08
Geomjeongkong 1	0.57	0.55	0.38	0.41	14.9	0.13
Hwaeomputkong	0.32	0.48	0.38	0.28	13.2	0.09
Pureunkong	0.42	0.41	0.45	0.33	13.5	0.1
Hannamkong	0.48	0.40	0.65	0.42	10.3	0.1
CV (%)	19.8	11.6	13.3	15.4	-	-
LSD (0.05)	0.14	0.09	0.1	0.1	-	-

the lowest (3176.1 μg g^{-1}) and the differences between the varieties were significant. Daweonkong also had the highest total phenol contents (0.60%) at storage for one year, two years (0.80%), and four years (0.44%). However, it did not have the highest total phenol contents for three years. The variation of total phenol content with storing years was not significant for Taekwangkong, but was significant for both Daweonkong and Hwaeomputkong (Table 2 and 4). Analyzing the results with regard to colored seed coats, the phenol content of black soybeans (5279.4 μg g^{-1}) was higher than

Table 3. Composition of 11 individual phenolic compounds in soybean varieties stored for four years.

Phenolics Variety	Storing years	Gentisic acid	*p*-hydroxy benzoic acid	Chlorogenic acid	Caffeic acid	Syringic acid	*p*-coumaric acid	Ferulic acid	Hesperidin	Narigin	Salicylic acid	Hyrictin	Total
		μg g^{-1}											
Muhankong	1	410.9	31.1	nd	nd	nd	9.2	nd	nd	nd	333.1	nd	784.2
	2	753.4	45.8	nd	0.9	nd	31.2	1.6	31.1	4.7	1249.2	42.8	2160.6
	3	328.9	36.6	nd	nd	nd	31.7	0.8	17.5	7.2	661.6	22.4	1106.6
	4	504.2	62.4	nd	1.0	0.5	26.9	1.7	1.3	4.8	291.6	26.1	920.4
Daweonkong	1	32.9	51.9	3.9	nd	2.1	4.2	nd	nd	nd	265.4	19.4	379.6
	2	391.4	60.5	7.5	0.5	5.6	22.5	1.7	24.5	13.8	1034.2	28.7	1590.9
	3	191.3	32.7	0.9	nd	1.5	31.5	1.7	12.0	1.5	557.2	26.3	856.6
	4	113.3	74.3	2.3	0.9	3.8	13.8	1.7	42.1	1.9	181.2	20.4	455.9
Myeongju namulkong	1	344.9	43.9	nd	nd	nd	14.2	nd	nd	nd	819.2	19.7	1241.8
	2	560.5	29.4	1.3	0.6	4.6	33.8	nd	14.6	1.2	1181.4	nd	1827.4
	3	349.3	22.1	2.3	0.5	0.1	56.9	nd	17.2	9.2	1032.5	19.9	1509.7
	4	342.7	33.4	2.1	0.9	0.3	63.7	2.1	3.0	1.0	813.2	20.3	1282.6
Jinpumkong 2	1	448.0	nd	nd	nd	nd	8.3	nd	nd	nd	362.9	nd	819.2
	2	1072.6	48.4	2.3	1.1	0.2	42.1	0.8	29.5	2.7	1185.6	20.0	2405.2
	3	327.4	19.0	2.0	nd	nd	37.7	nd	1.8	8.6	576.0	9.6	982.1
	4	511.6	49.2	2.1	1.0	0.5	36.3	2.4	5.6	5.8	415.6	19.1	1049.0
Taekwangkong	1	415.4	34.4	Nd	nd	nd	10.7	nd	nd	nd	295.9	9.5	765.8
	2	750.7	43.5	Nd	nd	0.1	24.0	nd	18.5	1.0	745.2	20.5	1603.4
	3	219.3	21.1	2.2	nd	0.1	2.7	1.5	2.4	3.6	201.2	19.4	473.5
	4	226.2	35.4	Nd	nd	nd	19.5	nd	1.9	2.7	183.9	9.8	479.4
Geomjeongkong 1	1	392.3	35.4	Nd	1.0	nd	5.1	nd	nd	nd	197.2	9.4	640.3
	2	704.5	57.0	1.0	0.5	1.1	19.6	1.5	18.5	12.3	687.5	23.7	1527.2
	3	281.4	18.4	Nd	nd	0.1	0.8	nd	5.4	1.2	280.8	19.5	607.6
	4	185.3	57.5	0.9	0.4	0.5	19.9	1.7	2.8	2.5	148.8	23.4	443.7
Hwaeomputkong	1	255.2	34.1	Nd	nd	nd	6.6	nd	nd	nd	208.3	19.3	523.5
	2	361.4	56.8	Nd	0.5	0.4	7.7	nd	9.0	1.7	240.2	19.7	697.3
	3	588.5	50.5	nd	nd	0.2	6.8	nd	1.9	0.9	134.6	9.6	793.0
	4	96.0	41.1	1.1	nd	0.1	8.5	nd	1.5	0.7	89.5	nd	238.4
Pureunkong	1	377.2	45.3	nd	nd	nd	21.5	1.6	nd	nd	581.1	nd	1027.9
	2	1046.9	64.5	2.4	nd	0.1	50.3	1.6	29.0	3.6	1280.3	23.2	2501.8
	3	590.4	49.5	1.1	0.5	1.5	44.7	1.6	1.5	10.7	492.4	nd	1193.8
	4	310.3	66.9	nd	Nd	0.2	47.0	1.8	2.2	8.5	497.8	19.8	954.4
Hanamkong	1	396.6	53.3	1.2	Nd	nd	16.5	nd	18.8	1.2	627.7	23.0	1137.0
	2	681.7	64.5	1.6	2.8	0.4	30.1	0.8	28.5	4.5	1143.2	43.2	2001.2
	3	42.5	45.9	nd	1.0	0.3	35.2	0.8	4.2	0.9	277.1	9.8	800.7
	4	334.4	58.8	nd	1.1	0.5	15.2	1.6	2.1	7.7	276.1	19.0	746.4
LSD (0.05)	1	58.3	6.4	2.2	0.1	0.4	1.5	0.1	0.5	0.1	0.1	43.5	14.6
	2	698.6	36.6	2.5	2.1	7.8	25.5	1.2	20.8	4.7	922.9	27.9	1709.1
	3	175.8	15.8	1.6	0.7	1.6	8.4	1.2	10.7	8.7	95.5	18.0	281.0
	4	335.4	13.3	1.5	0.5	0.5	7.5	0.6	7.3	3.9	89.9	11.1	409.5

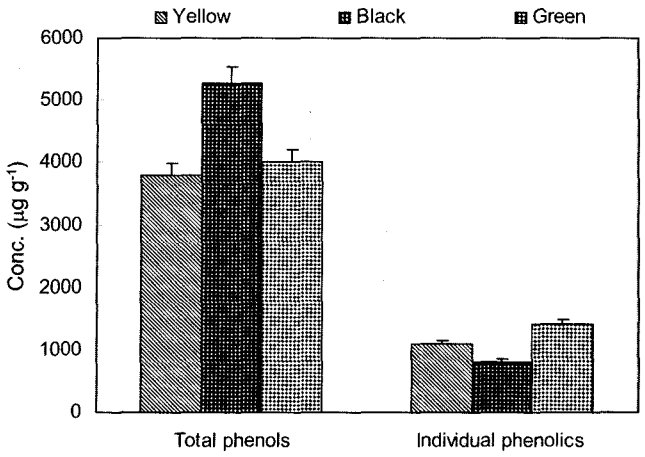

Fig. 3. Composition of phenolics content among different in soybean seeds with colored seed coats (Vertical bars are represented of the standard error values).

Table 4. Composition of total phenol contents and individual phenolic compounds in the nine soybean varieties.

Varieties	Total phenol contents	Total mean of individual phenolic compounds
	μg g^{-1}	
Muhankong	3176.1	1243.0
Daweonkong	5789.8	820.7
Myeongjunamulkong	3545.4	1465.4
Jinpumkong 2	3651.5	1313.9
Taekwangkong	3793.6	830.5
Geomjeongkong 1	4768.9	804.7
Hwaeomputkong	3661.0	563.0
Pureunkong	4005.7	1419.5
Hannamkong	4869.3	1171.3
CV (%)	23.72	55.4
LSD(0.05)	795.6	592.4

yellow (3782.8 μg g^{-1}) and green soybeans (4005.7 μg g^{-1}). There were significant differences between each colored seed coat (Fig. 3).

An analysis of total phenol contents using the Folin-Dennis method showed a variation from 0.05 to 0.67% in sorghum due to factors such as different varieties, colored seed coat and maturation time (Bate-Smith *et al.*, 1969). Maxon & Rooney (1972) reported that the total phenol contents of sorghum grain (0.40 to 0.64%) were affected by the test procedure, standard chemicals and extraction method. Lee & Lee (1994) reported that soybean seeds varied from 0.28 to 0.32% in total phenol content using tannic acid as a calibration curve. However, these values were lower than the total phenol contents in this study (0.26 to 0.80%).

In this study, variation of total phenol content was significant and slightly correlated to storage over four years. Environmental factors in each cropping year had no affect on total phenol contents, except for temperature in July (r^2=0.22*) (correlation data not provided).

These facts suggest that over long storing periods at room temperature, chemicals such as phenol compounds in soybean seeds oxidize. It was also considered that absolute comparisons of total phenol contents between other studies were not appropriate because of the effect of using different standard chemicals and extraction methods.

In the future, we will focus on studying the role of storing years and determining better extraction methods than those currently used for the analysis of total phenol contents.

Variation of 11 individual phenolic compounds for four storing years

In general, phenolic compounds are chemicals with high antioxidant and allelopathic properties. These chemical actions are related to a number of hydroxyl (-OH) functional groups among the phenolic compounds (Chen & Ho, 1997). Total contents of 11 phenolic compounds were significant over storage for four years when measured by HPLC, means of total concentration were highest (1812.8 μg g^{-1}) at storage for two years and lowest (730.0 μg g^{-1}) at storage for four years (Fig. 2). Myeongjunamulkong had the highest mean of phenolic concentration (1465.4 μg g^{-1}) of all varieties and Hwaeomputkong had the lowest (563.0 μg g^{-1}) (Table 3, 4 and Fig. 4). At storage for one year, Myeongjunamulkong had the highest total phenolic concentration (1241.8 μg g^{-1}) and Daweonkong had the lowest (379.6 μg g^{-1}). Gentisic acid (344.9 μg g^{-1}) and salicylic acid (819.2 μg g^{-1}) contents were high in Myeongjunamulkong. Some minor components of some varieties were not completely detected, or were detected in very small amounts, and these compounds are included with hesperidin, narigin and syringic acid. At storage for two years, total phenolic contents were highest in Pureunkong (2501.8 μg g^{-1}) and lowest in Hwaeomputkong (697.3 μg g^{-1}). Gentisic acid and salicylic acid were also major compounds, while ferulic acid, syringic acid and chlorogenic acid were only detected in very small amounts in the nine varieties (Table 3). At storage for three years, three varieties, including Pureunkong (1193.8 μg g^{-1}), had high phenolic contents over 1000.0 g^{-1}, with Myeongjunamulkong (1509.7 μg g^{-1}) having the highest contents overall. On the other hand, Taekwangkong (473.5 μg g^{-1}) had the lowest contents because gentisic acid (219.3 μg g^{-1}) and salicylic acid (201.2 μg g^{-1}) were detected in very small amounts compared with the other varieties. Caffeic acid was only detected in three varieties, including Hannamkong, while ferulic acid and chlorogenic acid were found in only

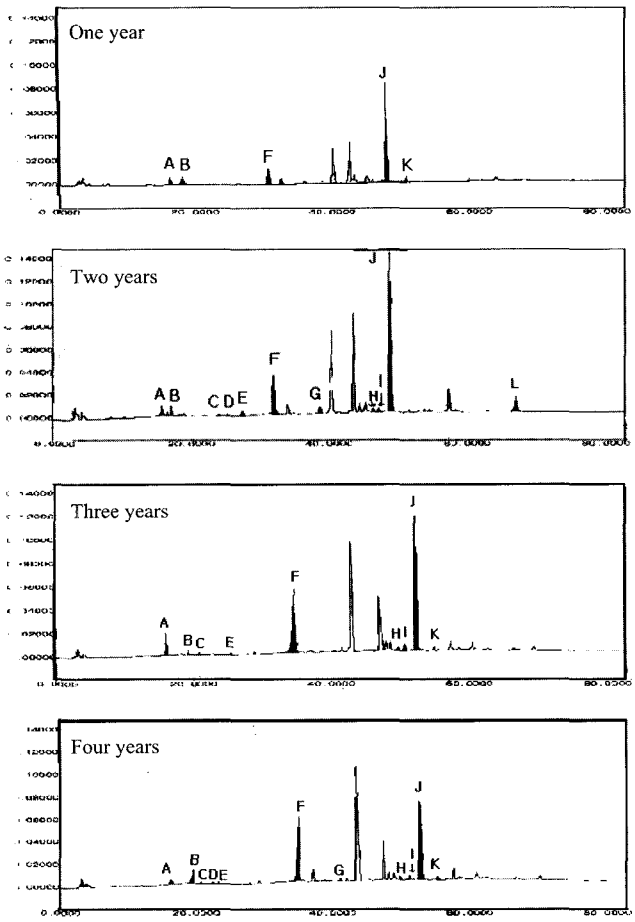

Fig. 4. Variation of individual phenolics concentrations in Myeongjunamul stored for four years. A: gentisic acid, B: catechin, C: chlorogenic acid, D: caffeic acid, E: syringic acid, F: *p*-coumaric acid, G: ferulic acid, H: hesperidin, I: narigin, J: salicylic acid, K: hyricetin, L: naringenin.

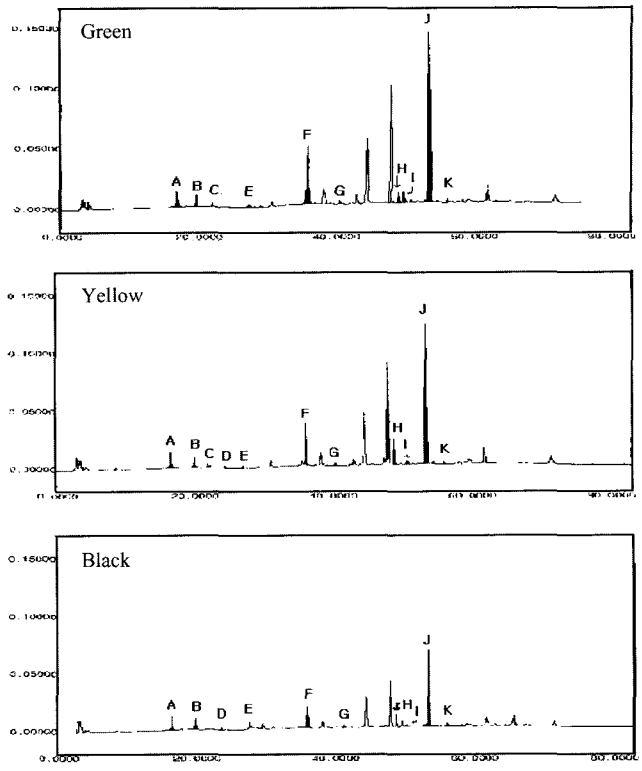

Fig. 5. Composition of individual phenolics in soybean seeds stored for two years. A: gentisic acid, B: catechin, C: chlorogenic acid, D: caffeic acid, E: syringic acid, F: *p*-coumaric acid, G: ferulic acid, H: hesperidin, I: narigin, J: salicylic acid, K: hyricetin, L: naringenin.

very small amounts (Table 3). At storage for four years, Myeongjunamulkong (1282.6 μg g^{-1}) and Jinpumkong 2 (1049.0 μg g^{-1}) had high quantities over 1000.0 μg g^{-1}, while four varieties, including Hwaeomputkong (238.4 μg g^{-1}), had low contents under 500.0 μg g^{-1}. The major phenolic compounds in Myeongjunamulkong and Jinpumkong 2 were gentisic and salicylic acids. Chlorogenic acid, caffeic acid, syringic acid and ferulic acid were not completely detected or detected in only very small amounts in the nine varieties (Table 3). In addition, there were differences in the total phenolic contents of green (1419.5 μg g^{-1}), yellow (1097.8 μg g^{-1}) and black-coated soybeans (812.7 μg g^{-1}). There were significant differences with colored seed coat and individual phenolic compounds in storage over several years (Fig. 3 and 5).

The contents of phenolic among total phenol have been measured at 28% in defatted soybean seeds (Seo & Morr, 1984). In this study, the ratio of phenolic compounds to total phenol contents varied from 20.96 to 47.73% in storage for four years. This difference may be due to practices such as limited standard chemicals or different pre-treatments of the soybean samples. Bae *et al.* (1997) reported that *p*-coumaric acid comprised 44 to 56% of the total phenolic compounds in soybean varieties, while salicylic acid was only found in micro-quantities. However, Pratt *et al.* (1982) reported that *p*-coumaric acid was found in very small amounts and the total content of phenolic compounds differed in each study (Bae *et al.*, 1997). Gentisic and salicylic acids were found in only micro-quantities in soybean seeds. However, they were detected in large quantities for all varieties in this study. Different pre-treatments, including extraction and analysis methods using HPLC, may explain the differences between these studies. The total contents of individual phenolic compounds varied from 238.4 to 2501.8 μg g^{-1} with varieties for storage over four years, although there was little variation for Hwaeomputkong.

Total contents were negatively correlated with storing years (r^2=-0.2*), but were highly positively correlated with gentisic (r^2=0.88***) and salicylic acids (r^2=0.95***). According to Tsai & Todd (1972) and Tevini *et al.* (1983), drought condition in the cropping years could lead to noticeably decreased

levels of cinnamic and benzoic acid derivatives in wheat, although they had no effect in the radish. Generally, it is thought that a decrease of phenolic compounds might result from a decline in the activity of key enzymes in the biosynthesis of phenolic compounds (Bardzik *et al.*, 1971). On the other hand, the cucumber was induced to increase phenolic compounds under water stress. However, the total contents of 11 phenolic compounds were not related to environmental factors in the cropping years such as temperature, precipitation and day length, although temperature in July (r^2=0.25*) was slightly positively related with total phenolic compounds (correlation data not provided). Although a little precipitation in September 2001 might have resulted in a decrease in phenolic compounds in that year compared with the others, precipitation was not found to affect phenolic contents in this study. Hence, variations of the eleven individual phenolic compounds are thought to be slightly more related to storing years than to genotype, methods of cultivation and unknown environmental factors.

Thus, future studies will focus on the effect of storing years on changes in phenol contents in soybean seeds and in the improvement of storing environments.

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