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ORIGINAL PAPER

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Analysis of phenolic compounds and isoflavones in soybean seeds (*Glycine max* (L.) Merill) and sprouts grown under different conditions

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Abstract Soybeans (*Glycine max* (L.) Merill) are popularly known as a healthy food in many Asian countries and are mostly consumed as soymilk, tofu, and fermented products such as miso, temph, and sufu. The objective of this study was to determine the variation and composition of phenolic compounds and isoflavone contents in soybean seeds [*Glycine max* (L.) Merill] and sprouts [Kongnamul] grown under dark conditions (producing yellow soybean sprouts) and in green and yellow boxes (producing green soybean sprouts). In seven soybean cultivars, the total phenolic content ranged from 6.67 μg^{-1} in Pureunkong to 72.33 μg^{-1} in Poongsannamulkong. The average total phenolic content in the green soybean sprouts (48.33 μg^{-1}) was higher than in the yellow soybean sprouts (29.75 μg^{-1}). The total phenolic content in the yellow soybean sprouts varied from 9.88 μg^{-1} to 47.71 μg^{-1}, and the total phenolic content in the green soybean sprouts varied from 29.21 μg^{-1} to 79.70 μg^{-1}. Only four phenolic compounds, *p*-hydroxybenzoic acid, salicylic acid, *p*-coumaric acid, and ferulic acid, were detected in all soybean cultivars. Syringic acid was not detected in yellow soybean sprouts, and myricetin was only detected in yellow soybean sprouts (4.65 μg^{-1}) from the Pureunkong cultivar grown under dark conditions. The total isoflavone content in soybean seeds ranged from 2.1 μg^{-1} in Sowonkong to 33.0 μg^{-1} in Pureunkong, and the mean total isoflavones was 10.61 μg^{-1}. Green soybean sprouts had higher average total isoflavones (1389.4 μg^{-1}) than yellow soybean sprouts (559.2 μg^{-1}), and the total isoflavone content was highest in the Pureunkong yellow soybean sprouts (756.3 μg^{-1}) and the Sowonkong green soybean sprouts (2791.6 μg^{-1}). In soybean sprouts, the higher the (malonyl)-daidzin or (malonyl)-genistein content, the higher the total isoflavone level. Our study suggests that producing soybean sprouts enriched in isoflavones under coloured-light sources is feasible.

Keywords Isoflavones · Phenolic compounds · Soybean seeds · Soybean sprouts · Green and yellow boxes · HPLC analysis

Introduction

Soybeans [*Glycine max* (L.) Merill] are one of the most important food crops in Korea. The benefits of soybean-based foods are well known and are widely recognized around the world [1]. Recently, demands for health foods have increased because of their many advantages for human health. Many countries use soybeans in various forms such as soybean sprouts, pastes, soymilk, soybean oil, and tofu as key ingredients in cultural cuisine.

Soybean sprouts [Kongnamul] is a principal food consumed in Korea, and recent demand for them has increased because of renewed interest in functional foods [2]. Soybean seeds contain many phenolic compounds such as chlorogenic acid, caffeic acid, ferulic acid, and *p*-coumaric acid. These have antioxidant effects that are beneficial to human health [3–6]. Generally, these phenolic acids have contents ranging from 28 to 72% [7] of the total phenol level in soybean seeds. Mega and Lorenz [8] reported individual phenolic acid contents in defatted soybean seeds of 256 μg^{-1} using high performance liquid chromatography (HPLC). Other studies have isolated *p*-hydroxybenzoic (139 μg^{-1}), *p*-coumaric (90 μg^{-1}), and *t*-caffeic acids

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($60\ \mu g^{-1}$) from soybean seeds [9, 10]. According to several studies, the analysis of phenolic compounds is usually performed with HPLC, and syringic acid, ferulic acid, and vanillic acid have been found to be the main components in soybean seeds [8, 11–13].

A form of flavonoid in soybean seeds, isoflavones, has been found to have important secondary compounds with many chemical actions. Examples include antioxidative actions, and functions as anticancer agents [3, 4, 14]. Isoflavones are categorized chemically by their functional groups. There are four subgroups: aglycons, glucosides, malonylglucosides, and acetylglucosides [15]. Isoflavones found in soy products are well-known phytooestrogens because they appear to have oestrogen-like activity and structure [16–18]. According to many studies, isoflavones are essential for preventing certain forms of cancer, as well as reducing the risk of cardiovascular diseases [17, 19–27]. Recent literature studies indicate that isoflavones have benefits against cardiovascular diseases and cancers, and function by acting as anti-oestrogens [28, 29]. More specifically, two forms of isoflavones, genistein and daidzein, have been found to be the major isoflavones in soybean seeds. They have the ability to control or inhibit the growth of human breast cancer cell lines in culture. In particular, genistein possesses the strongest antioxidative properties [9, 30–34]. Many studies have also reported correlations between isoflavone content and cultivation environment, which includes different locations and temperatures [35–37]. Isoflavone contents are generally affected by environmental and genetic factors such as cultivation year, soybean cultivar, cultivation location, and temperature. Isoflavone contents also vary with storage time and germination period.

In general, light, including light quality, intensity, and duration, has pronounced influences on the germination, growth, and development of plants, as well as in the formation of secondary metabolites [38]. The effect of light on the production of some secondary plant metabolites such as anthocyanins, flavonoids, anthraquinones, and alkaloids, has been investigated by focusing on light-mediated enzyme regulation of culture cells. However, little information is available regarding the effect of light on isoflavones and phenolic compounds in soybean sprouts. This is despite germinated soybean sprouts being a form frequently consumed in East Asia. The main purposes of this study were: (1) to compare the isoflavones and phenolic contents in soybean seeds and soybean sprouts grown under dark and light conditions and (2) to develop a new soybean sprout cultivation method that produces higher isoflavone and phenolic contents.

Materials and methods

Samples

The seven soybean [*Glycine max* (L.) Merill] cultivars used in this experiment were cultivated at Iksan in 2003 (Honam Agricultural Research Institute, RDA, Korea). Appropriate pesticides were used to manage weeds, diseases, and insects; and fertilizers were applied before ploughing at the recommended rates of 8, 8, and 12 kg per 1000 m^2 for N, P_2O_5, and K_2O, respectively. Soybean seeds were harvested from three replications of each cultivar for a cropping year and stored at room temperature until analyses of phenolic compounds and isoflavones were conducted in 2005.

Soybean sprout cultivation

Soybean seeds were soaked in distilled water at room temperature (25 °C) for 4 h before being cultivated as sprouts. Seeds cultivated under dark conditions in a greenhouse for 5 days produced only yellow soybean sprouts. This form of cultivation is a popular standard method used in Korea (Fig. 1). Soybean seeds grown under two different box conditions produced only green soybean sprouts. Each box was manufactured from two differently coloured cellophane tapes and an acryl film (case size: 30 cm×30 cm×45 cm). First, soybean seeds were cultivated in the dark for 1 day until they germinated. On days 2 and 3, the soybean sprouts were placed in a green box under a light source. Finally, on days 4 and 5, they were moved to a yellow box under the same light source (Fig. 1). Both yellow and green sprouts were cultivated under controlled temperature (25 °C), and the conditions were replicated three times. This new cultivation method for producing green soybean sprouts with high levels of secondary metabolites has been patented by our research group in the Korea [38].

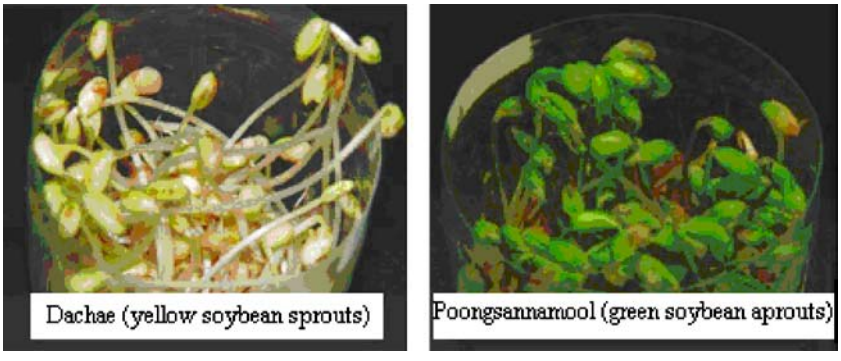

Fig. 1 Yellow and green soybean sprouts

Preparation of soybean samples for HPLC analysis

Samples (2 g) including whole soybean seed were ground, mixed with 10 ml of acetonitrile and 2 ml of 0.1 N HCl, and stirred for 2 h at room temperature. The solution was filtered through a filter paper (Whatman no. 42), and the sample was then dried in a freeze dryer (temperature below −30 °C). Finally, it was redissolved in 10 ml of 80% methanol (HPLC grade). Redissolved samples were filtered through 0.45 um micro-filter paper, and then transferred to 2 ml vials. These samples were then used to analyze phenolic compounds and isoflavones.

HPLC analysis of phenolic compounds

The HPLC system incorporated a Young-Lin M930 liquid chromatograph pump and an M720 detector. The column for analysis was a YMC-Pack ODS-AM-303 (250 mm×4.6 mm I.D.), and UV absorption was measured at 280 nm. Instrumentation for HPLC analysis was applied using the method of Kim et al. [39]. In the HPLC analysis, the mobile phases consisted of solvents A and B. Solvent A was 98% water and 2% glacial acetic acid in 0.018 M ammonium acetate. Solvent B was 70% solvent A and 30% organic solvent, which consisted of 82% methanol, 2% glacial acetic acid in 0.018 M ammonium acetate, and 16% *n*-butanol. The following gradient was used: 0.0–1.0 min isocratic at 10% B, 1.0–21.0 min linear gradient from 10 to 25% B, 21.0–36.0 min linear gradient from 25 to 45% B, 36.0–56.0 min linear gradient from 45 to 100% B, 50.00–50.15 min flow increased to 1.20 ml min^{-1}, 82.00–82.15 min linear gradient from 100 to 10% B, 92.00–92.15 min flow decreased to 1.00 ml min^{-1}, and at 99.00 min the sampled loop was rinsed and the gradient repeated [11, 39]. The 12 phenolic compound standards were purchased from Sigma Aldrich (USA) and were used for calibration curves.

HPLC analysis of isoflavones

Instrumentation for HPLC analysis was applied using the method of Hoeck et al. [36]. The soybean samples used for the analysis of isoflavones were the same as those for the phenolic compounds. In the HPLC analysis, the mobile phase consisted of solvents A and B. Solvent A was 0.1% glacial acetic acid in distilled water, and solvent B was 0.1% glacial acetic acid in ACN. The injection volume was 20 ul of the samples. Solvent B was increased from 15 to 35% over 50 min, and then held at 35% for 10 min. The flow rate was 1 ml min^{-1}. The isoflavone standards were purchased from Sigma Aldrich (USA) and were used for calibration curves. Daidzein, genistein, glycitein, daidzin, genistin, glycitin, acetyldaidzin, acetylgenistin, acetylglycitin, malonyldaidzin, malonylgenistin, and malonylglycitin were each identified by their retention times.

Statistical analyses

Analyses of variance were performed using the general linear model procedure of the SAS program (SAS Institutes, Inc., 2000). All the aforementioned experiments were replicated three times using a completely randomized design. Pooled mean values were separated on the basis of least significant difference (LSD) at the 0.05 probability level.

Results and discussion

Comparisons of phenolic compounds in soybean seeds and yellow and green soybean sprouts

Phenolic compounds are generally secondary metabolites with high antioxidative and antiaging properties. Their activities *in vitro* and *in vivo* are related to a number of hydroxyl functional groups in their structures [37]. Total phenolic compounds in the soybean seeds ranged from 6.67 μg^{-1} in Pureunkong to 72.33 μg^{-1} in Poongsannamulkong. Although we conducted quantitative analyses of 12 phenolic compounds using HPLC, only four phenolic compounds (*p*-hydroxybenzoic acid, salicylic acid, *p*-coumaric acid, and ferulic acid) were detected in the soybean seed.

Mean salicylic acid content (10.9 μg^{-1}) was the highest of all phenolic compounds. However, it was only detected in three of the seven cultivars and its content varied significantly. The ferulic acid content was low in the soybean seeds and varied significantly between the cultivars. *p*-Hydroxybenzoic acid and *p*-coumaric acid were detected in all cultivars. The *p*-hydroxybenzoic acid content (11.63 μg^{-1}) was highest in Dachaekong, and the *p*-coumaric acid content (13.27 μg^{-1}) was highest in Sowonkong (Table 1, Fig. 2).

Table 1 Composition of phenolic compounds in soybean seeds

Cultivar	*p*-Hydroxybenzoic acid (μg^{-1})	Salicylic acid (μg^{-1})	*p*-Coumaric acid (μg^{-1})	Ferulic acid (μg^{-1})	Total (μg^{-1})
Daweonkong	3.34	nd	5.58	0.93	9.85
Pureunkong	1.90	nd	3.94	0.83	6.67
Seonamkong	2.65	nd	5.58	1.73	9.96
Paldokong	1.66	nd	5.01	0.71	7.38
Poongsannamoolkong	3.31	52.65	2.48	13.89	72.33
Dachaekong	11.63	21.59	3.58	nd	36.80
Sowonkong	2.22	2.18	13.27	0.97	18.64
$LSD_{(0.05)}$	0.03	0.12	0.03	0.08	3.78

nd: not detected

Table 2 shows the distribution of 12 phenolic compounds in soybean sprouts grown under dark and coloured light conditions. The average total phenolic content was higher in green soybean sprouts (48.33 μg^{-1}) than in yellow soybean sprouts (29.75 μg^{-1}) (Figs. 2 and 3). Among the yellow soybean sprouts, Paldokong (47.71 μg^{-1}) had the highest total phenolic content and Daweonkong (9.88 μg^{-1}) had the lowest. Salicylic acid (8.44 μg^{-1}) and naringenin contents (28.91 μg^{-1}) were highest in Paldokong yellow soybean sprouts, and the ferulic acid content (21.49 μg^{-1}) was highest in Pureunkong yellow soybean sprouts. Variations differed significantly between soybean cultivars. Syringic acid was not detected in any soybean cultivar grown under dark conditions, and myricetin was only detected in Pureunkong yellow soybean sprouts (4.65 μg^{-1}) grown in the dark.

Among the green soybean sprouts, Sowonkong (79.70 μg^{-1}) had the highest total phenolic content and

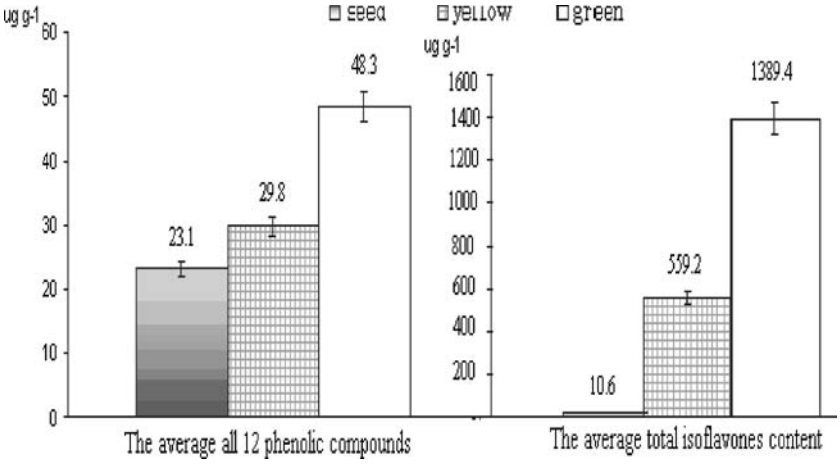

Fig. 2 Comparisons between average total phenolics and isoflavone content in soybean sprouts grown under different cultivation conditions

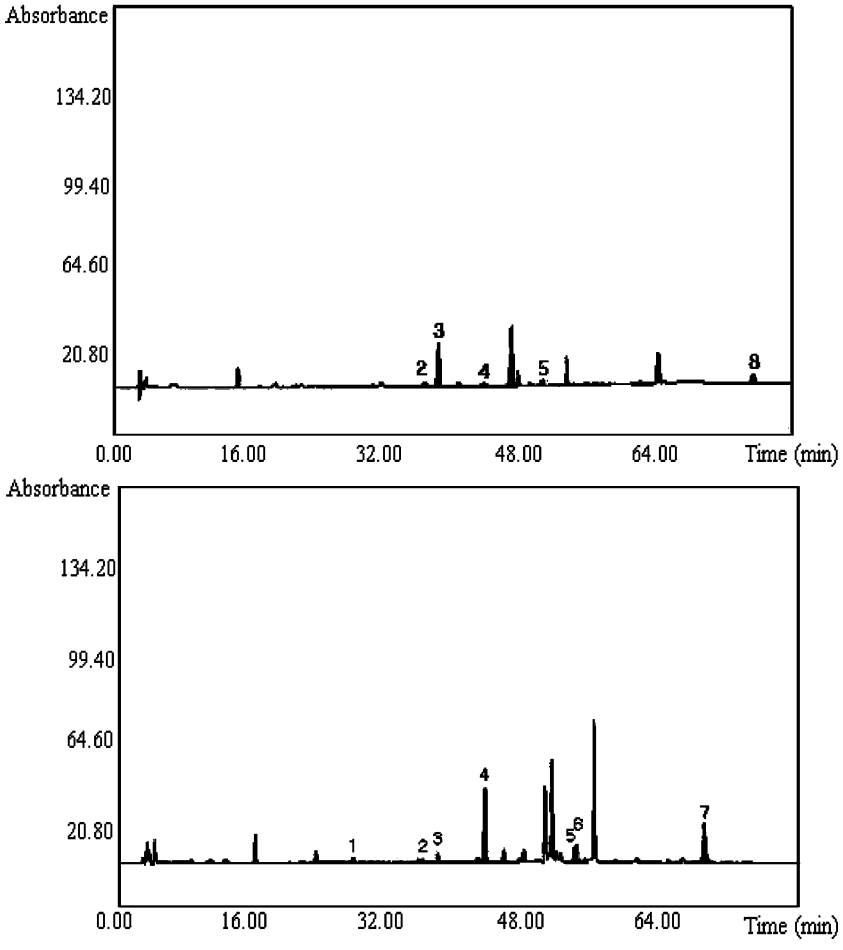

Fig. 3 HPLC chromatograms of phenolic compounds in soybean sprouts (*above*: yellow soybean sprouts, *below*: green soybean sprouts, 1: caffeic acid, 2: salicylic acid, 3: *p*-coumaric acid, 4: ferulic acid, 5: naringin, 6: hesperidin, 7: *trans*-cinnamic acid, 8: naringenin)

Table 2 Composition of 12 phenolic compounds in yellow and green soybean sprouts

	Chemical (μg^{-1})													
	Cultivar	1	2	3	4	5	6	7	8	9	10	11	12	Total
D[a]	G[h]	nd	nd	0.17	0.94	17.02	0.33	2.16	2.07	3.61	4.18	2.02	nd	32.50
	Y[i]	nd	nd	0.49	nd	2.12	0.29	2.71	0.63	1.90	nd	1.74	nd	9.88
	$LSD_{(0.05)}$	–	–	0.06	0.04	0.06	0.06	0.06	0.06	0.06	0.04	0.06	–	1.34
P[b]	G	nd	nd	1.47	0.19	5.28	1.32	2.39	3.23	4.23	nd	9.42	1.68	29.21
	Y	nd	nd	1.62	nd	4.84	1.50	21.49	3.35	6.09	4.65	nd	0.64	44.18
	$LSD_{(0.05)}$	–	–	0.06	0.04	0.06	0.06	0.06	0.06	0.06	0.04	0.04	0.06	5.79
S[c]	G	nd	1.44	1.14	nd	4.13	1.44	14.91	6.16	8.67	4.09	8.35	0.72	51.05
	Y	nd	nd	nd	nd	3.69	0.82	10.59	3.52	5.68	nd	2.76	nd	27.06
	$LSD_{(0.05)}$	–	0.6	0.04	–	0.06	0.06	0.06	0.06	0.06	0.04	0.06	0.04	8.76
Pa[d]	G	nd	8.55	0.30	0.26	1.89	0.32	26.17	8.85	12.09	5.75	5.09	0.89	70.16
	Y	3.49	0.37	nd	nd	8.44	4.99	1.51	nd	nd	nd	nd	28.91	47.71
	$LSD_{(0.05)}$	0.04	0.06	0.04	0.04	0.06	0.06	0.04	0.04	0.04	0.04	0.04	0.06	4.70
Ps[e]	G	nd	0.95	1.20	nd	6.59	1.64	10.14	2.39	3.35	nd	4.83	nd	31.09
	Y	nd	nd	0.37	nd	5.28	1.17	8.02	2.32	4.22	nd	1.47	nd	22.85
	$LSD_{(0.05)}$	–	0.02	0.06	–	0.06	0.06	0.06	2.19	0.06	–	0.06	–	11.50
Da[f]	G	nd	nd	nd	nd	5.57	1.50	16.97	5.05	6.73	3.73	5.07	nd	44.62
	Y	nd	nd	nd	nd	1.59	4.64	0.95	0.14	nd	nd	nd	14.27	21.59
	$LSD_{(0.05)}$	–	–	–	–	0.06	0.06	0.06	0.06	0.04	0.04	0.04	0.04	5.34
So[g]	G	nd	1.83	3.43	0.14	5.85	4.41	31.87	8.57	8.67	5.15	8.57	1.21	79.70
	Y	nd	nd	1.61	nd	2.75	1.60	18.43	4.12	6.72	nd	2.44	nd	37.67
	$LSD_{(0.05)}$	–	0.04	0.06	0.04	0.06	0.06	0.06	0.06	0.06	0.04	0.06	0.04	7.99

1: *p*-Hydroxybenzoic acid, 2: chlorogenic acid, 3: caffeic acid, 4: syringic acid, 5: salicylic acid, 6: *p*-coumaric acid, 7: ferulic acid, 8: naringin, 9: hesperidin, 10: myricetin, 11: *trans*-cinnamic acid, 12: naringenin

[a]Daweonkong, [b]Pureunkong, [c]Seonamkong, [d]Paldokong, [e]Poongsannamoolkong, [f]Dachaekong, [g]Sowonkong, [h]green soybean sprouts, [i]yellow soybean sprouts, LSD: 0.05 probability level

nd: not detected

Pureunkong (29.21 μg^{-1}) had the lowest. When grown under green and yellow boxes, chlorogenic acid (8.55 μg^{-1}), hesperidin (12.09 μg^{-1}), and myricetin (5.75 μg^{-1}) contents were highest in the Paldokong cultivar, and caffeic acid (3.43 μg^{-1}) was highest in the Sowonkong cultivar. The content of salicylic acid (17.02 μg^{-1}) was highest in Daweonkong green soybean sprouts grown under the same green and yellow boxes (Table 2).

The proportion of phenolic acids among the total phenol compounds, which are measured using Folin-Dennis methods, varied from 20.96 to 47.73%. Variations in their contents are affected by cultivar, test method, and environmental stresses such as drought and temperature [7, 39]. Total phenolic contents differ between studies. For example, *p*-coumaric acid was found to vary between 44 and 56% of the total phenolic compounds in soybean seeds in the study by Bae et al. [40], while Pratt et al. [9] only detected a very small amount of *p*-coumaric acid in soybean seeds. Kim et al. [39] reported that the total phenolic content was highly correlated with amounts of gentisic acid (r^2=0.88***) and salicylic acid (r^2=0.95***). These facts support our result that ferulic acid and salicylic acid were higher in yellow and green soybean sprouts. In addition, Kim et al. [39] reported a range in content from 730.0 μg^{-1} to 1812.8 μg^{-1} in 11 phenolic compounds during storage over 4 years. They also found that the total phenolic content was not related to environmental factors such as temperature and precipitation of the cultivation year, although temperature in July (r^2=0.25*) showed a slightly positive correlation with the total phenolic content.

In this study, we detected a very low total phenolic content and believe that the 2-year storage time before analysis may have been responsible. Phenolic compounds varied significantly in seeds with different coloured seed coats (data not shown). The average total phenolic content was generally higher in yellow-coated soybean seeds (29.03 μg^{-1}) than in those with black (9.87 μg^{-1}) or green (6.69 μg^{-1}) seed coats. However, these results differed slightly from those of Kim et al. [39], who noted a higher average total phenolic content in green-coated seeds (1419.5 μg^{-1}) than in those with yellow (1097.8 μg^{-1}) or black (812.7 μg^{-1}) seed coats. These differences are believed to have resulted from environmental factors or different cultivation locations.

In our study, the light conditions under which the soybean sprouts grew was the only parameter that changed. The total phenolic content in green soybean sprouts was higher than in yellow soybean sprouts, and this was due to the coloured light that penetrated through the yellow and green boxes. The different coloured lights that the soybean sprouts received increased the total phenolic content by promoting photosynthesis and the malonyl CoA pathway, which is related to the synthesis of phenolic compounds in the soybean sprouts. In future, we will

continue to study correlations between the synthesis of phenolic compounds and different coloured-light sources. We will also try to explain why green soybean sprouts are more effective at synthesizing phenolic compounds, using genetic engineering or labelled ^{14}C or^{13}C techniques.

Comparison of isoflavone content in soybean seeds and yellow and green soybean sprouts

Isoflavones are antioxidants that are one of the most important secondary metabolites in plants. They are called phytooestrogens because they are similar to the female hormones called "oestrogens" [1]. In this study, we carried out a quantitative analysis of seven soybean varieties and their sprouts by growing them in either green then yellow, or dark boxes. In the soybean seeds, the isoflavone content ranged from 2.1 μg^{-1} in Sowonkong to 33.0 μg^{-1} in Pureunkong, and the mean total isoflavones of the seven cultivars was 10.61 μg^{-1}. In addition, out of the four isoflavone subgroups, only glucoside- and malonyl-conjugated groups were detected. Of note, HPLC detected a small amount of glucoside- and malonyl-conjugated groups in three of the seven cultivars (Table 3, Fig. 2).

Under the two different light conditions, the mean total isoflavones and malonyl-conjugated groups were higher in green than in yellow soybean sprouts. The average total isoflavone content in the green soybean sprouts was 1389.4 μg^{-1}, and the content of the malonyl-conjugated groups was 1034.3 μg^{-1}. In the yellow soybean sprouts, the average total isoflavone content was 559.2 μg^{-1}, and the content of the malonyl-conjugated groups was 313.9 μg^{-1}. The content of aglycon groups was low, even in the green soybean sprouts (Fig. 2).

Among the yellow soybean sprouts grown under the dark condition, Pureunkong had the highest total isoflavone content (756.3 μg^{-1}), and the lowest was found in Daweonkong (216.2 μg^{-1}). Of the four isoflavone subgroups in the yellow soybean sprouts, one of the malonyl-conjugated groups, malonylgenistin, had the highest content in Pureunkong (226.6 μg^{-1}). In the glucosides group, the genistin content was highest in Sowonkong (95.9 μg^{-1}). In the aglycons group, the daidzein content was highest in Paldokong (157.6 μg^{-1}) (Table 4, Figs. 4 and 5).

In the green soybean sprouts, Sowonkong (2791.6 μg^{-1}) had the highest total isoflavones, and Daweonkong (527.2 μg^{-1}) had the lowest. Of the soybean cultivars, Sowonkong had the highest content of malonylgenistin (1241.2 μg^{-1}). In the glucoside groups, daidzein and genistein were usually high in all cultivars. However, Pureunkong (176.9 μg^{-1}) and Seonamkong (144.7 μg^{-1}) were especially high in daidzein content (aglycons) compared with the other cultivars. The above results were very similar for yellow soybean sprouts grown under dark conditions (Table 4, Figs. 4 and 5).

Isoflavone contents in soybean seeds generally have large variations that depend on genetic characteristics and other factors such as commercial processes in an industry, and environmental factors such as location, temperature, and so on. Eldridge and Kwolek [41] reported that variations in total isoflavones ranged from 1176 to 3309 μg^{-1} in soybean varieties grown at the same location. Wang and Murphy [35] noted that the isoflavone content in seven American soybean varieties ranged from 2053 to 4216 μg^{-1}. Effects of the cultivation year had a much greater influence on the variation in isoflavone content than the location. Kim et al. [15] and Tsukamoto et al. [42] reported that a

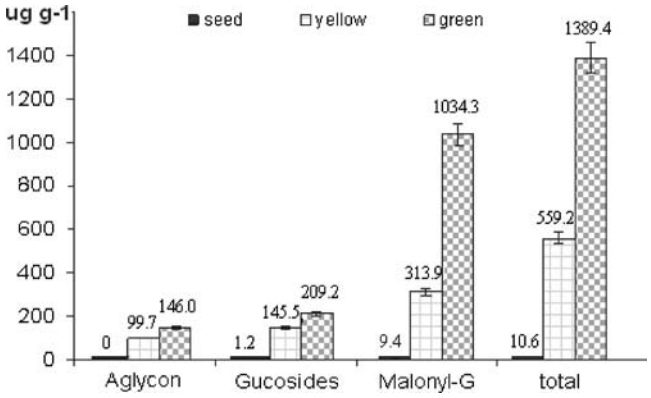

Fig. 4 Comparisons between the average isoflavone content of isoflavone groups

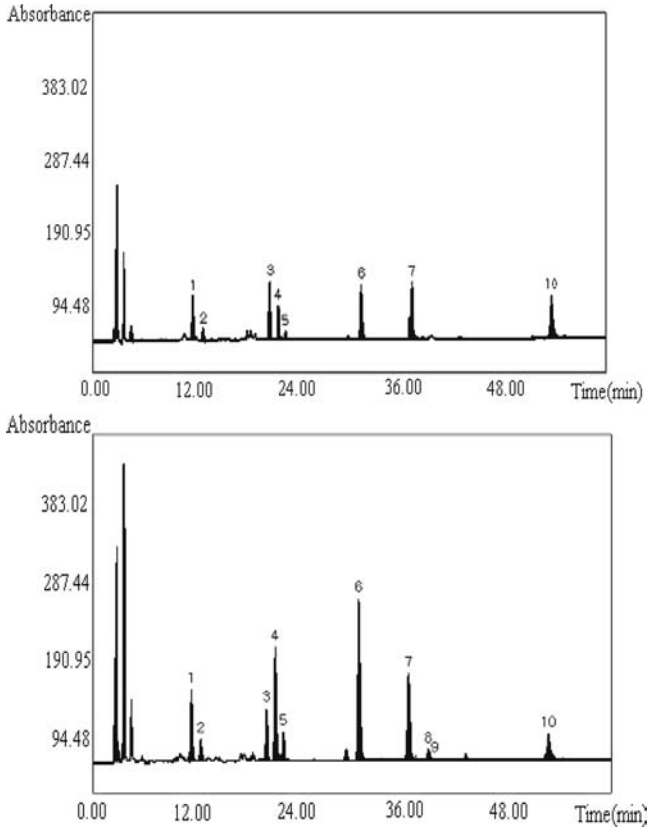

Fig. 5 HPLC chromatograms of isoflavone content in soybean sprouts (*above*: yellow soybean sprouts, *below*: green soybean sprouts, 1: daidzin, 2: glycitin, 3: genistin, 4: malonyldaidzin, 5: malonylglycitin, 6: malonylgenistin, 7: daidzein, 8: glycitein, 9: acetylgenistin, 10: genistein)

Table 3 Composition of isoflavones in soybean seeds

Cultivar	Glucoside (μg^{-1})			Malonylglucoside (μg^{-1})			Total (μg^{-1})
	Din	Gly	Gin	Din	Gly	Gin	
Daweonkong	0.1	tr	1.2	15.6	tr	1.7	18.6
Pureunkong	1.3	tr	3.1	20.7	tr	7.9	33.0
Seonamkong	0.2	tr	1.6	16.1	tr	2.7	20.6
Paldokong	tr	tr	tr	tr	tr	tr	–
Poongsannamoolkong	tr	tr	tr	tr	tr	tr	–
Dachaekong	tr	tr	tr	tr	tr	tr	–
Sowonkong	tr	tr	0.7	tr	tr	1.4	2.1
$LSD_{(0.05)}$	0.25	–	0.25	0.25	–	0.28	0.28

[a]Daidzin,[B]glycitin,[C]genistin
tr: trace

Table 4 The composition of isoflavone contents of yellow and green soybean sprouts

Cultivar		Glucoside ($\mu g\ g^{-1}$)			Malonylglucoside ($\mu g\ g^{-1}$)			Aglycon ($\mu g\ g^{-1}$)			Total ($\mu g\ g^{-1}$)
		Din[a]	Gly[b]	Gin[c]	Din	Gly	Gin	Dein[d]	Gein[e]	Glein[f]	
Daweonkong	G[g]	9.9	3.7	29.7	145.8	17.6	289.0	22.4	6.1	3.0	527.2
	Y[b]	13.5	4.3	20.7	67.2	13.5	67.3	23.1	6.7	–	216.2
	LSD	1.5	0.8	2.8	5.1	0.6	13.1	1.5	0.8	0.3	14.3
Pureunkong	G	73.1	4.1	102.7	522.1	35.3	582.3	176.9	88.9	64.2	1649.6
	Y	77.4	12.3	89.6	196.8	15.7	226.6	98.1	24.7	15.1	756.3
	LSD	25.3	3.2	35.8	174.3	11.2	204.5	64.8	35.1	30.9	573.1
Seonamkong	G	96.6	54.2	78.3	446.9	117.6	391.6	144.7	38.4	15.1	1383.4
	Y	53.2	27.4	47.5	152.8	41.2	115.3	32.3	9.36	–	479.1
	LSD	16.6	4.8	6.8	12.6	2.9	16.8	5.2	0.9	0.1	52.9
Paldokong	G	69.2	41.2	66.7	474.9	147.3	495.5	38.7	15.4	3.2	1352.1
	Y	54.3	29.5	88.4	115.3	33.3	157.1	157.6	98.5	18.1	752.1
	LSD	25.2	11.5	23.9	84.3	35.8	135.9	277.2	150.6	53.5	501.1
Poongsan-namoolkong	G	56.7	9.49	82.4	143.9	16.3	208.1	77.7	4.9	21.7	621.2
	Y	58.9	4.5	86.9	138.4	18.5	152.5	26.2	10.5	–	496.4
	LSD	24.1	14.7	3.9	8.2	13.3	17.5	5.4	1.4	0.1	52.1
Dachaekong	G	113.3	33.5	109.8	485.6	85.1	455.5	59.6	56.8	1.8	1401.0
	Y	57.7	18.1	78.6	122.9	24.7	133.9	83.3	48.9	2.2	570.3
	LSD	16.3	1.8	11.9	22.7	5.3	22.7	22.6	17.6	0.7	104.5
Sowonkong	G	160.4	60.1	209.1	864.8	73.8	1241.2	117.6	45.7	18.9	2791.6
	Y	77.7	22.1	95.9	175.9	29.9	198.7	31.9	9.7	1.8	643.6
	LSD	22.5	10.3	47.5	123.6	9.1	169.3	17.8	5.6	2.3	376.9

[a]Daidzin,[b]glycitin,[c]genistin,[d]daidzein,[e]genistein,[f]glycitein,[g]green soybean sprout,[h]yellow soybean sprout

short storage time prevents the degradation of isoflavones over time. During seed development, the isoflavone content was also significantly higher at low temperature. However, the boiling, milling, and protein coagulation processes in tofu production do not significantly destroy daidzein or genistein. In contrast, other methods such as roasting (high heat treatment) resulted in a 15–21% loss of daidzein and genistein [43]. Toda et al. [43] reported that glucoside conjugates within the isoflavone subgroups are converted to isoflavone aglycons during the manufacturing process by β-glucosidase. However, in this study, the total isoflavones in soybean seeds were low relative to other studies. It is thought that the isoflavones in the seeds decreased during the 2 years of storage time under uncontrolled temperature and humidity before they were grown into soybean sprouts. The low total isoflavones may also have been affected by high temperatures or the drought experienced during the 2003 cultivation year.

In Korea, soybean sprouts are usually cultivated under dark conditions. However, sprouts grown in the dark have a lower overall nutritional value than sprouts grown under coloured light. This general trend also pertains to isoflavones, and results from the lack of photosynthesis in the dark box. Isoflavones are synthesized in the malonate and phenylpropanoid pathways using three malonyl CoAs and one coumaroyl CoA, which are produced during photosynthesis [44]. Therefore, it is possible to control the light conditions in order to increase the amount of malonyl CoA and coumaroyl CoA produced during the photosynthesis process. This, in turn, would increase the isoflavone content in soybean sprouts. In this study, we have applied a new cultivation method using coloured light. A light source penetrates through green and yellow boxes, allowing the sprouts to receive coloured light. Soybean sprouts grown under this type of light produce green soybean sprouts, which contain increased amounts of health-promoting isoflavones.

In this study, we demonstrated that the light could trigger the formation of plant phytochemicals in soybean sprouts. In addition, green soybean sprouts showed a green colour throughout, and it is thought that the whole sprout contains more chlorophyll to perform photosynthesis. On the basis of our results, we suggest that the application of coloured lights, controlled by films, is an effective and useful method for producing highly nutritional and functional foods that are promising candidates with antioxidative and anticarcinogenic potential. Receiving a patent with this lighting technique indicates that this method can effectively control isoflavone synthesis in soybean sprouts [38]. However, no clear relationship between the lighting colour and isoflavone content has been established. Thus, more studies should be undertaken to examine increases or fluctuations in these substances under controlled light.

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References

1. Holt S (1997) Korean Soybean Digest 14:77–90
2. Kim YH, Hwang YH, Lee HS (2003) Korean J Food Sci Technol 35(4):568–575
3. Kim SH, Jung WS, Ahn JK, Chung IM (2005) Eur Food Res Technol 220:207–214
4. Chung IM, Kim KH, Ahn JK, Chi HY, Lee JO (2000) Korean J Crop Sci 45:328–334
5. Naim M, Benjamin G, Aron B, Yehudith B (1976) J Agric Food Chem 22:806–811
6. Hayes RE, Bookwalter GN, Bagley EB (1997) J Food Sci 6:1527–1532
7. Seo A, Morr CV (1984) J Agric Food Chem 32:530–536
8. Mega JA, Lorenz K (1974) J Sci Agr 25:797–802
9. Pratt DE, Birac PM (1979) J Food Sci 44:1720–1727
10. Dabrowski KJ, Sosulski FW (1984) J Agric Food Chem 32:128–130
11. Barnwart WL, Porter PM, Granyo TC, Hassett JJ (1985) J Chem Ecol 11:383–395
12. Wieslaw O, Lee CY, Antoni W, Price KR (1988) J Agric Food Chem 36:430–432
13. Stainslaw B, Wieslaw O, Lee CY (1990) J Agric Chem 38:945–948
14. Kim JJ, Kim SH, Hahn SJ, Chung IM (2005) Food Res Intern 38:435–444
15. Wang HJ, Murphy PA (1994) J Agric Food Chem 42:1666–1673
16. Coward L, Barnes NC, Setchell KDR, Barnes S (1993) J Agric Food Chem 31:392–396
17. Anderson JW, Johnstone BM, Cook-Newell ME (1995) New Engl J Med 333:276–282
18. Umpress ST, Murphy SP, Franke AA, Custer LJ, Blitz CL (2005) J Food Compost Anal 18:533–550
19. Graber M, June CH, Samelson LE, Weiss A (1992) Int Immunol 4:1201–1210
20. Fortis T, Pepper M, Adlercreutz H, Fleischmann G, Hase T, Montesano R, Schweigerer R (1993) Proc Natl Acad Sci USA 90:2690–2694
21. Jing YK, Nakaya K, Han R (1993) Anticancer Res 13:1049–1054
22. McCabe MJ, Orrenius S (1993) Biochem Biophys Res Commun 194:944–950
23. Mousavi Y, Adlercreutz H (1993) Steroids 58:301–304
24. Pagliacci MC, Spinozzi F, Migliorati G, Fumi G, Smacchia M, Grignani F, Riccardi C, Nicoletti I (1993) Eur J Cancer 29:1573–1577
25. Messina M, Persky V, Setchell K, Barnes S (1994) Nutr Cancer 21:113–131
26. Anthony MS, Clarkson TB, Hughes CL, Morgan TM, Burke GL (1996) J Nutr 126:43–50
27. Schultz WB (1998) Fed Reg 63:62977–63015
28. Adlercreutz H, Barnwart C, Wahala K, Makela T, Brunow G, Hase T, Arosemena PJ, Kellis JT, Vickery Jr LE (1993) J Steroid Biochem Mol Biol 44:147–153
29. Cassidy A (1996) Proc Nutr Soc 55:399–417
30. Choi YB, Sohn HS (1998) Korean J Food Sci Technol 30(4):745–750
31. Peterson G, Barnes S (1991) Biochem Biophys Res Commun 179:661–667
32. Kwoon TW, Song YS, Hong JH, Moon GS, Kim JI, Hong JH (1998) Korea Soybean Digest 15:1–12
33. Jha HC, Von Recklinghausen G, Zilliken F (1985) Biochem Pharmacol 34:1367
34. Kusonoki T, Higashi H, Hosai S, Hata D, Sugie K, Mayumi M, Migawa H (1992) Biochem Biophys Res Commun 183:789–796
35. Wang HJ, Murphy PA (1994) J Agric Food Chem 42:1674–1677
36. Hoeck JA, Fefr WR, Murphy PA, Grace AW (2000) J Agric Food Chem 40:48–51
37. Chen HC, Ho CT (1997) J Agric Food Chem 45:2374–2378
38. Ro JS, Chung IM (2005) Method for manufacturing green elemental bean sprouts using yellow light. Patent No. 428997, Korea
39. Kim SH, Song HK, Ahn JK, Kim JT, Hahn SJ, Chung IM (2004) Korean J Crop Sci 49:82–88
40. Bae EA, Kwon TW, Lee YS, Moon GS (1997) Korean Soybean Digest 14:12–20
41. Eldridge AC, Kwolek W (1983) J Agric Food Chem 31:394–396
42. Tsukamoto S, Shimada S, Ignite K, Kudou S, Kikuyu M, Okubo K (1995) J Agric Food Chem 43:1184–1192
43. Toda T, Sakamoto A, Takayanagi T, Yokotsuka K (2001) Food Sci Tech Res 6:314–319
44. Heldt HW (2005) Plant Biochem (3rd edn) *Elsevier Acdemic Press*. Amsterdam