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Yearly and Genotypic Variations in Seed Isoflavone Content of Local Soybean Cultivars

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ABSTRACT : To analyze their isoflavones contents by HPLC analysis during two years on 60 collected old local traditional soybean varieties from various districts of Korea was conducted. There was a yearly variation in the seed isoflavon concentrations. The total contents on 5 isoflavones were in the range from 16.21 mg/g to 25.21 mg/g and from 6.47 mg/g to 15.44 mg/g, in 1997 and 1998, respectively. Collected soybean from Gunsansi-1 (25.21 mg/g) in 1997 and from Gangjingun-3 (16.50 mg/g) in 1998 showed the highest amount of isoflavones as compared with other collected soybean varieties. The highest amount among 5 isoflavones was genistin as 48.45% and 49.73%, in 1997 and 1998, respectively, indicating the genotypic variation in seed isoflavon contents of local soybean cultivar. Our data suggest that it may be feasible for improving soybean variety with higher antioxiadtive activity and substances.

Keywords **:** soybean, isoflavones, HPLC, variety, yearly variation

Soybean (*Glycine max* (L.) Merrill), one of the most principal upland food crops in Korea, was domestically consumed over 1.423 M/T in 2000. Only 116,000 tons were domestically produced with the self-supply rate of less than 8.1% (Jo, 2000). To copy with the current status of domestic and international soybean production and consumption, domestically produced soybean should be distinct from imported ones by its advanced quality and diversification. Also, the health benefits of food made with soybean have been well known for a long time and have been significantly recognized in the world (Holt, 1997; Kyoko, 1998). Nowadays, demand for having so called "health food" is increasing in many countries. Soybean provides potentially beneficial effects for several of the most common disorders that afflict human beings, including cancer *et al.* (Holt, 1997).

Isoflavones are one of the significant component in soybean. The major isoflavones, genistein and daidzein, and minor one, glycitein, in soybean inhibit the growth of human breast cancer and prostate cancer cell lines in culture (Choi *et al.*, 1996). The isoflavones was transformed into another chemical substance by some functional group such as glucoside, the malonyl glucoside and the acetyl glucoside (Wang and Murphy, 1994a, b). Especially genistein has attracted a great deal of attention as it is one of the major isoflavones in soybean (Kwon *et al.*, 1998). Daidzein, the other main isoflavone present in soybean, had also reported to retain antiestrogenic activity (Coward *et al.*, 1993; Peterson and Barnes, 1991). These isoflavones compounds in soybean has been known as antihemolytic, antioxidative, antifungal, and antitumorial activity (Kudou *et al.*, 1991; Chung *et al.*, 2000). Although several researchers had investigated isoflavones contents in the fermented soybean, unfermented soybean, Korea traditional fermented foods and the role of the genotype, environment, and genotype x environment interactions on isoflavone content of soybean (Choi and Sohn, 1998; Choi *et al.*, 1996; Kim *et al.*, 1996; Coward *et al.*, 1993; Wang and Murphy, 1994a,b; Hoeck *et al.*, 2000; So *et al.*, 2001), little information is available on breeding soybean variety with high antioxidative substances.

The main purpose of this study was to analyze the amount of 5 isoflavones (daidzin, genistin, glycitein, daidzein, genistein) using high performance liquid chromatography (HPLC) on collected local old traditional soybean in Korea. Results of this study may provide basic informations for breeding soybean variety with higher antioxidative activity and isoflavones substances.

MATERIALS AND METHODS

Materials

Sixty varieties of soybean (*Glycine max* (L.) Merrill) which were 27 collected soybean from Jeollanamdo, 14 collected soybean from Jeollabukdo, and 19 collected soybean from Chungcheongnamdo were cultivated and harvested in experimental field, College of Agriculture and Life Science, Konkuk University, in 1997 and 1998 as described previously study (Chung *et al.*, 2000). The harvested sample was

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kept in a cool chamber at below -35°C until used for this study.

Isoflavones extraction for HPLC analysis

The ground soybean seeds (2 g) including seed coat were mixed with 2 mL of 0.1N HCl and 10 mL of acetonitrile, stirred for 2 hrs at room temperature, and filtered through Whatman No. 42 filter paper. The filtrate was taken to dryness under vacuum a temperature below 30°C. The dried material was redissolved in 10 mL of 80% HPLC grade MeOH in distilled water. A aliquot of sample was filtered through a 0.45 m filter unit and analyzed by HPLC. Authentic standards of daidzin, daidzein, genstin, and genstein were obtained from commercial source (Aldrich Chemical Co.). The glycitein was also obtained with some modifications by the method of Wang and Murphy (1994b).

HPLC analysis of isoflavones

The instrumentations for HPLC analysis were applied by the method of Wang and Murphy (1994a, b). A linear HPLC gradient was employed: solvent A was 0.1% glacial acetic acid in distilled H_2O, and solvent B was 0.1% glacial acetic acid in acetonitrile. Following injection of 20 μL of sample, solvent B was increased from 15% to 35% over 50 min and then held at 35% for 10 min. The solvent flow rate was 1 mL/min. The HPLC system used Yon-Lin company and the column employed YMC-AM 303 (ODS 4.52×50 mm). The UV detector wavelength was 254 nm. Standard compounds were chromatographed alone and as mixture. HPLC standard chromatogram which was mixed five isoflavones standard was gained. Relative retention times for the major peaks in the extracts were determined for each gradient by dividing the compound's retention time by the retention time of an internal reference.

Table 1. Isoflavone contents on 60 collected soybean varieties in 1997.

Collected varieties	Din[a†]	Gin[b]	Glen[c]	Dein[d]	Gein[e]	Total	Collected varieties	Din[a]	Gin[b]	Glein[c]	Dein[d]	Gein[e]	Total
		mg/g							mg/g				
Damyang-gun	8.92	9.73	0.59	1.17	0.68	21.09	Jeongeup-si-2	5.08	9.75	g0.64	0.71	0.73	16.91
Hampyeoung-gun	6.55	8.03	1.44	1.37	0.64	18.03	Iksan-si-1	8.41	10.20	0.61	1.23	0.69	21.14
Hwasun-gun	7.66	10.02	0.54	1.12	0.61	19.95	Iksan-si-2	5.77	9.76	0.59	1.12	1.03	18.27
Gangjin-gun-1	5.38	10.13	0.99	0.81	0.75	18.06	Imsil-gun	8.61	9.26	0.44	1.16	0.63	20.10
Gangjin-gun-2	5.84	9.39	0.60	0.86	0.75	17.44	Jinan-gun	4.06	9.62	0.99	0.70	0.84	16.21
Gangjin-gun-3	8.10	10.69	0.54	1.21	0.73	21.27	Kunsan-si-1	11.44	7.56	0.48	4.46	1.17	25.21
Gohung-gun	3.63	10.06	0.75	0.92	0.94	16.30	Kunsan-si-2	6.80	9.45	1.42	1.34	0.88	19.89
Gurye-gun-1	3.93	9.97	1.37	0.58	0.75	16.60	Namwon-si-1	6.05	8.58	3.03	1.07	0.66	19.39
Gurye-gun-2	6.98	9.66	1.20	1.22	0.80	19.86	Namwon-si-2	10.03	11.78	0.56	1.40	0.68	24.47
Gwangyang-si-1	5.89	7.82	1.37	0.98	0.65	16.71	Okgu-gun	8.04	9.41	0.55	1.28	0.70	19.98
Gwangyang-si-2	6.27	9.02	3.39	0.93	0.57	20.18	Sunchang-gun	8.47	8.99	0.65	1.76	0.90	20.77
Gwangyang-si-3	6.98	8.29	0.41	1.24	0.72	17.64	Asan-si	7.76	8.13	0.68	1.86	0.81	19.24
Mokpo-si	9.02	7.41	0.63	1.59	0.62	19.27	Boryeong-si-1	4.72	8.71	1.09	0.89	0.78	16.19
Suncheon-si-1	5.91	9.72	0.57	0.69	0.62	17.51	Boryeong-si-2	7.62	9.10	0.74	1.01	0.47	18.94
Suncheon-si-2	5.12	8.47	1.49	0.79	0.67	16.54	Cheonan-si	7.96	8.43	0.53	1.25	0.72	18.89
Yeocheon-si-1	6.82	9.45	0.70	1.42	0.65	19.04	Gongju-si-1	9.10	8.76	0.63	1.36	0.69	20.54
Yeocheon-si-2	6.93	11.08	1.68	0.86	0.55	21.10	Gongju-si-2	8.09	10.21	0.51	1.36	0.78	20.95
Yeocheon-si-3	5.82	7.36	2.84	0.75	0.45	17.22	Geumsan-gun	8.64	8.79	0.65	1.18	0.59	19.85
Yeocheon-si-4	5.68	8.05	3.14	0.70	0.48	18.05	Nonsan-si	8.70	8.58	0.54	1.52	0.63	19.97
Yeocheon-si-5	5.60	10.51	0.67	0.80	0.71	18.29	Asan-si-1	6.75	11.19	0.50	0.69	0.62	19.75
Yeocheon-si-6	8.36	9.34	1.10	1.74	0.93	21.47	Asan-si-2	8.32	10.98	0.54	1.37	0.96	22.17
Yeocheon-si-7	6.51	8.34	1.84	1.09	0.64	18.42	Buyeo-gun-1	7.65	10.55	0.48	0.93	0.56	20.17
Yeosu-si-1	5.85	10.68	1.96	0.80	0.75	20.04	Buyeo-gun-2	7.58	10.55	2.60	1.47	0.97	23.17
Yeosu-si-2	5.18	9.29	1.62	0.95	0.91	17.95	Seocheon-gun-1	7.96	8.55	0.72	1.55	0.78	19.56
Yeosu-si-3	5.74	9.35	0.99	1.21	0.96	18.25	Seocheon-gun-2	7.88	7.86	0.84	1.26	0.56	18.40
Yeongam-gun	7.70	9.55	0.78	0.97	0.59	19.59	Seosan-si-1	8.34	8.51	0.74	1.09	0.49	19.17
YeongGawang-gun	5.09	9.48	1.20	0.80	0.79	17.36	Seosan-si-2	6.09	7.98	2.13	1.13	0.62	17.95
Jangsu-gun-1	7.46	9.90	0.68	1.42	0.79	20.25	Taean-gun	8.28	9.58	0.97	1.24	0.67	20.74
Jangsu-gun-2	7.39	11.96	0.55	1.12	0.90	21.92	Yesan-gun-1	7.14	9.74	1.51	0.82	0.58	19.79
Jongup-si-1	6.88	10.82	1.07	0.62	0.96	20.35	Yesan-gun-1	8.03	8.57	0.72	1.11	0.50	18.93
							CV(%)	2.81	2.07	8.97	8.74	3.66	1.70
							LSD(0.05)	0.34	0.33	0.15	0.18	0.04	0.53

[†a]; daidzin, [b]; genistin, [c]; glycitein, [d]; daidzein, [e]; genistein.

Statistical analysis

Analysis of variance for all data was accomplished using the general linear model procedure of the SAS program and all of the above mentioned experiments were replicated three times in a completely randomized design. The pooled mean values were separated on the basis of least significant difference (LSD) at the 0.05 probability (SAS Institute, 1985).

RESULTS AND DISCUSSION

HPLC analysis for isoflavones

Table 1 and 2 show the analysis of isoflavones in the collected sixty soybean. The total content of isoflavones showed the range from 16.21 mg/g to 25.21 mg/g and from 6.47 mg/g to 15.44 mg/g, in 1998. There was a significant difference in total isoflavone and individual isoflavone concentrations according to the cropping year. These are supported by the HPLC chromatogram of isoflavones (Fig. 2 and 3). This results were similar to those of Wang and Murphy (1994a,b) that isoflavones contents were influenced by genetic variation, crop years, growth location and condition. In this study, the environmental condition like climate may also be an attributing factor to the variation in the isoflavones contents (Table 3).

In the analysis of total isoflavones, collected soybean from Gunsansi-1 (25.21 mg/g), Namwonsi-2 (24.47 mg/g) and Buyeogun-2 (23.17 mg/g) showed the higher content as compared with other region collected soybean varieties in 1997 (CV=1.70%). In 1998 (CV=3.71%), however, collected soybean from Gangjingun-3 (16.50 mg/g), Gunsansi-2 (15.44 mg/g) and Yesangun-1 (14.57 mg/g) showed the higher content as compared with other region collected soybean varieties.

Table 2. Isoflavone contents on 60 collected soybean varieties in 1998.

Collected varieties	Din[a†]	Gin[b]	Glein[c]	Dein[d]	Gein[e]	Total	Collected varieties	Din[a]	Gin[b]	Glein[c]	Dein[d]	Gein[e]	Total
		mg/g							mg/g				
Damyang-gun	1.88	4.80	0.17	1.33	1.38	9.56	Jeongeup-si-2	1.88	4.80	0.17	0.33	0.38	7.56
Hampyeoung-gun	2.37	6.16	1.94	0.40	1.47	12.34	Iksan-si-1	4.23	6.39	0.20	1.15	0.65	12.62
Hwasun-gun	1.98	5.67	0.05	0.40	0.48	8.58	Iksan-si-2	4.13	4.84	0.15	1.05	0.66.	10.83
Gangjin-gun-1	2.27	4.65	2.44	0.59	0.65	10.60	Imsil-gun	3.85	5.75	0.16	1.80	1.01	12.57
Gangjin-gun-2	2.63	4.67	0.18	0.56	0.48	8.52	Jinan-gun	2.46	5.66	0.17	0.43	0.67	9.39
Gangjin-gun-3	5.62	8.90	0.09	1.08	0.81	16.50	Kunsan-si-1	3.08	7.20	0.10	0.34	0.42	11.14
Gohung-gun	1.46	4.24	0.14	0.28	0.35	6.47	Kunsan-si-2	4.30	7.52	0.72	1.40	1.50	15.44
Gurye-gun-1	1.85	4.28	3.82	0.33	0.56	10.84	Namwon-si-1	2.56	4.23	3.97	0.66	0.59	12.01
Gurye-gun-2	3.22	3.62	5.94	0.58	0.35	13.71	Namwon-si-2	3.76	5.79	0.05	0.61	0.34	10.55
Gwangyang-si-1	2.44	4.28	3.82	0.33	0.27	11.14	Okgu-gun	2.53	3.96	0.13	0.75	0.62	7.99
Gwangyang-si-2	2.50	4.01	4.73	0.55	0.31	12.10	Sunchang-gun	3.71	7.79	0.13	0.71	0.71	13.05
Gwangyang-si-3	3.15	3.35	0.06	0.58	0.24	7.38	Asan-si	3.42	7.19	0.09	0.39	0.39	11.48
Mokpo-si	3.49	4.35	0.11	0.71	0.32	8.98	Boryeong-si-1	2.09	4.15	0.50	0.39	0.39	7.52
SunchEon-si-1	2.39	4.07	0.14	0.36	0.28	7.24	Boryeong-si-2	2.04	5.59	0.06	0.38	0.52	8.59
SunchEon-si-2	2.38	3.93	1.86	0.43	0.37	8.97	Cheonan-si	4.08	6.23	0.11	0.60	0.39	11.41
Yeocheon-si-1	2.32	4.36	2.48	0.56	0.44	10.16	Gongju-si-1	4.43	5.27	0.05	0.94	0.57	11.26
Yeocheon-si-2	2.11	4.48	3.01	0.48	0.48	10.56	Gongju-si-2	3.81	5.97	0.07	0.41	0.42	10.68
Yeocheon-si-3	2.02	2.83	7.35	0.40	0.34	12.94	Geumsan-gun	2.78	7.11	0.07	0.37	0.38	10.71
Yeocheon-si-4	1.91	2.97	6.78	0.38	0.28	12.32	Nonsan-si	1.85	5.25	0.04	0.43	0.56	8.13
Yeocheon-si-5	2.77	5.96	0.12	0.76	0.74	10.35	Asan-si-1	3.23	6.37	0.20	1.08	0.92	11.80
Yeocheon-si-6	3.48	5.76	0.17	0.55	0.31	10.27	Asan-si-2	3.31	6.97	0.14	0.90	1.08	12.40
Yeocheon-si-7	3.71	5.88	1.33	0.91	0.81	12.64	Buyeo-gun-1	3.87	4.56	0.06	1.29	0.84	10.62
Yeosu-si-1	2.52	6.12	3.19	0.43	0.48	12.74	Buyeo-gun-2	3.13	7.05	2.28	0.57	0.59	13.62
Yeosu-si-2	2.47	4.01	0.29	0.28	0.36	7.41	Seocheon-gun-1	2.94	5.56	1.02	0.75	0.74	11.01
Yeosu-si-3	3.98	5.45	0.22	2.55	2.26	14.46	Seocheon-gun-2	2.98	6.84	0.14	0.32	0.36	10.64
Yeongam-gun	1.64	4.72	0.02	0.41	0.57	7.36	Seosan-si-1	1.76	4.56	0.02	0.48	0.65	7.47
YeongGawang-gun	2.52	3.76	2.00	0.52	0.48	9.28	Seosan-si-2	3.45	4.74	1.77	0.78	0.70	11.44
Jangsu-gun-1	4.67	6.63	0.03	0.64	0.37	12.34	Taean-gun	4.28	7.00	0.21	1.09	0.74	13.32
Jangsu-gun-2	3.41	6.46	1.27	1.08	0.88	13.10	Yesan-gun-1	4.14	6.28	2.27	0.90	0.98	14.57
Jongup-si-1	2.80	6.94	0.09	0.41	0.59	10.83	Yesan-gun-1	3.88	5.56	0.56	0.57	0.36	10.93
							CV(%)	7.81	4.18	8.01	18.03	5.61	3.71
							LSD(0.05)	0.38	0.36	0.15	0.19	0.05	0.65

[†a]; daidzin, [b]; genistin, [c]; glycitein, [d]; daidzein, [e]; genistein.

Table 3. Analysis of variance for soybean seed isoflavone content as affected by cropping year

Variety	Din[a†]	Gin[b]	Glein[c]	Dein[d]	Gein[e]	Variety	Din[a]	Gin[b]	Glein[c]	Dein[d]	Gein[e]
Damyang-gun	*‡	*	*	*	*	Jeongeup-si-2	*	*	*	*	*
Hampyeoung-gun	*	*	*	*	*	Iksan-si-1	*	*	*	*	*
Hwasun-gun	*	*	*	*	*	Iksan-si-2	*	*	*	*	*
Gangjin-gun-1	*	*	*	*	*	Imsil-gun	*	*	*	ns	*
Gangjin-gun-2	*	*	*	*	*	Jinan-gun	*	*	*	*	*
Gangjin-gun-3	*	*	*	*	*	Kunsan-si-1	*	*	*	*	*
Gohung-gun	*	*	*	*	*	Kunsan-si-2	*	*	*	*	*
Gurye-gun-1	*	*	*	*	*	Namwon-si-1	*	*	*	*	*
Gurye-gun-2	*	*	*	*	*	Namwon-si-2	*	*	*	*	*
Gwangyang-si-1	*	*	*	*	*	Okgu-gun	*	*	*	*	*
Gwangyang-si-2	*	*	*	*	*	Sunchang-gun	*	*	*	*	*
Gwangyang-si-3	*	*	*	*	*	Asan-si	*	*	*	*	*
Mokpo-si	*	*	*	*	*	Boryeong-si-1	*	*	*	*	*
SunchEon-si-1	*	*	*	*	*	Boryeong-si-2	*	*	*	*	*
Suncheon-si-2	*	*	*	*	*	Cheonan-si	*	*	*	*	*
Yeocheon-si-1	*	*	*	*	*	Gongju-si-1	*	*	*	*	*
Yeocheon-si-2	*	*	*	*	*	Gongju-si-2	*	*	*	*	*
Yeocheon-si-3	*	*	*	*	*	Geumsan-gun	*	*	*	*	*
Yeocheon-si-4	*	*	*	*	*	Nonsan-si	*	*	*	*	*
Yeocheon-si-5	*	*	*	ns	ns	Asan-si-1	*	*	*	*	*
Yeocheon-si-6	*	*	*	*	*	Asan-si-2	*	*	*	*	*
Yeocheon-si-7	*	*	*	*	*	Buyeo-gun-1	*	*	*	*	*
Yeosu-si-1	*	*	*	*	*	Buyeo-gun-2	*	*	*	*	*
Yeosu-si-2	*	*	*	*	*	Seocheon-gun-1	*	*	*	*	*
Yeosu-si-3	*	*	*	*	*	Seocheon-gun-2	*	*	*	*	*
Yeongam-gun	*	*	*	*	*	Seosan-si-1	*	*	*	*	*
YeongGawang-gun	*	*	*	*	*	Seosan-si-2	*	*	*	*	*
Jangsu-gun-1	*	*	*	*	*	Taean-gun	*	*	*	*	*
Jangsu-gun-2	*	*	*	*	ns	Yesan-gun-1	*	*	*	*	*
Jongup-si-1	*	*	ns	*	*	Yesan-gun-1	*	*	ns	*	*

†a; daidzin, b; genistin, c; glycitein, d; daidzein, e; genistein
‡ns, *Nonsignificant or Significant at 0.05 probability level, respectively.

In the analysis of individual isoflavone in 1997, collected soybean from Gunsansi-1 (11.44 mg/g) and Namwonsi-2 (10.03 mg/g) showed the highest daidzin contents, and collected soybean from Jangsugun-2 (11.96 mg/g), Namwonsi-2 (11.78 mg/g), and Asan-si-1 (11.19 mg/g) showed the higher genistin contents, and collected soybean from Gwangyangsi-2 (3.39 mg/g), Yeocheonsi-4 (3.14 mg/g) and Namwonsi-1 (3.03 mg/g) showed the higher glycitein contents, and collected soybean from Gunsansi-1 (4.46 mg/g) and Asansi (1.86 mg/g) exhibited the higher daidzein contents, collected soybean from Gunsansi-1 (1.17 mg/g) and Iksansi-2 (1.03 mg/g) exhibited the higher genistein contents.

Also, in the analysis of individual isoflavone in 1998, collected soybean from Gangjingun-3 (5.62 mg/g) and Jangsugun-1 (4.67 mg/g) showed the higher daidzin contents, and collected soybean from Gangjingun-3 (8.90 mg/g), Sunchanggun (7.79 mg/g), and Gunsansi-2 (7.52 mg/g) showed the higher genistin contents, and collected soybean from Yeocheonsi-3 (7.35 mg/g), Yeocheonsi-4 (6.78 mg/g), and Guryegun-2 (5.94 mg/g) exhibited the higher glycitein contents, and collected soybean from Yeosusi-3 (2.55 mg/g) and Imsilgun (1.80 mg/g) exibited higher daidzein contents, collected soybean from Yeosusi-3 (2.26 mg/g) and Gunsansi-1 (1.50 mg/g) exhibited the highter genistein contents. Results of this study are similar to those obtained by others (Choi *et al.*, 1996; Kwon *et al.*, 1998) who concluded that variation existed in isoflavones contents among varieties.

The highest isoflavone content among 5 isoflavones was genistin as 48.45% and 49.73%, in 1997 and 1998, respectively. This results were also similar to those of Murphy *et al.* (1997) who reported that the highest content among isoflavones was genistin, indicating that it may be possible

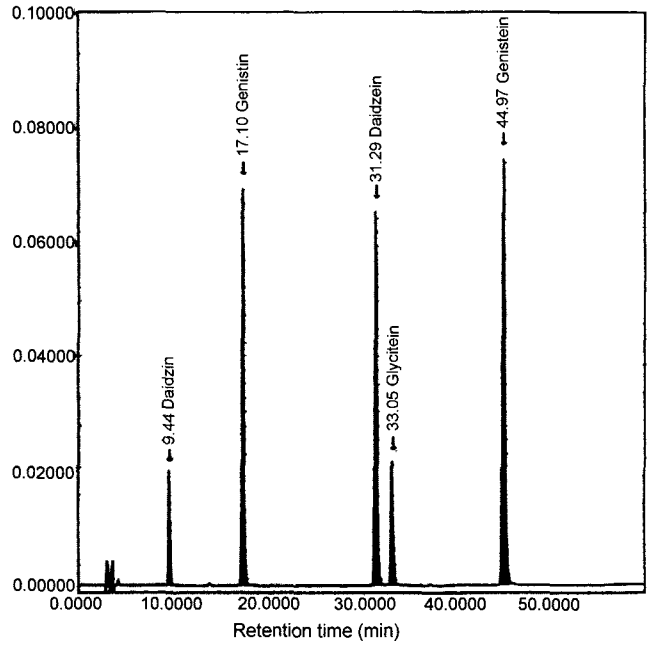

Fig. 1. HPLC chromatogram of standard chemical mixtures at 254 nm.

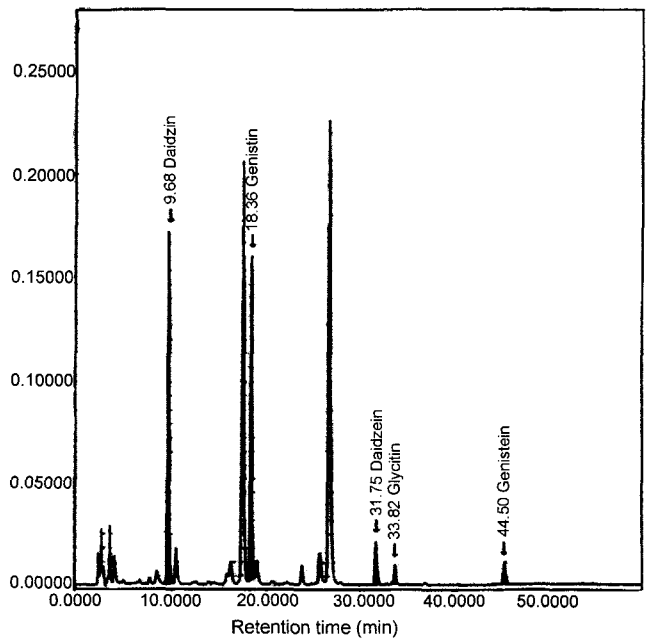

Fig. 2. HPLC chromatogram of collected soybean from Kangjingun-3 in 1997 at 254 nm.

to breed soybean variety containing higher antioxidative substances, isoflavones like genistin.

Fig. 1 shows HPLC chromatogram which was gained by five isoflavones standard mixtures. Fig. 2 and 3 represent HPLC analysis chromatograms of collected soybean at Gangjingun-3 in 1997 and 1998, respectively. The chromatogram of HPLC analysis showed many unknown peaks.

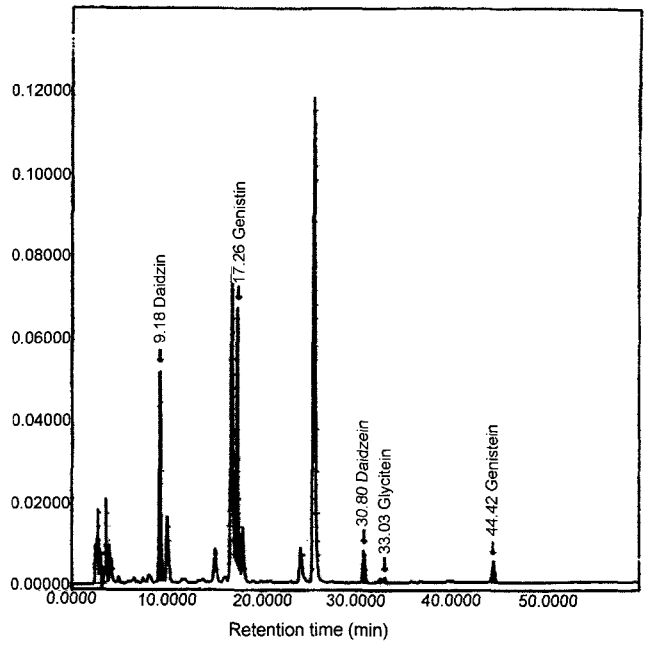

Fig. 3. HPLC chromatogram of collected soybean from Kangjingun-3 in 1998 at 254 nm.

This suggested that extracts contain a complicated mixture of other bioactive compounds except isoflavones. Thus, identification using proton nuclear magnetic resonance (NMR) need to identify unknown compounds.

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