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Changes in Isoflavone Contents during Maturation of Soybean Seed

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Abstract

Isoflavones are associated with prevention of chronic diseases such as cancer, osteoporosis, and hypercholesterolemia. The isoflavone contents at different development stages of soybean were analyzed to examine the period of their formation and reaching maximum level. Soybean seeds have been collected from 13th to 21st week after sowing. Total daidzein and genistein contents increased from 115 and 37mg/kg at 13th week, to 795 and 699mg/kg at 21st week, respectively. Total glycitein content showed less remarkable change during seed development than the other isoflavones. Thus mature soybean may have maximum preventive effect from chronic diseases since it contains the highest level of isoflavones.

Key words: isoflavones, genistein, glycitein, daidzein, maturation, soybean

INTRODUCTION

Soybeans and soy foods have been consumed abundantly by Koreans as a main dietary source of protein and fat(1). Current studies suggest that consumption of soybean-containing diets has been associated with the lower incidence of certain human diseases in Asians compared with Caucasian populations(2-5). Several studies attributed the physiologically active properties of soybeans to not only protein but isoflavones which are mainly present in the conjugated forms in the whole seed (6,7). Isoflavones including genistein, daidzein, and glycitein have been reported to have anticarcinogenic, antifungal, and antioxidant activities(2-5,8). In particular, genistein which is a potent tyrosine kinase inhibitor, has attracted much attention due to its possible role in preventing breast and prostate cancers(5,9-11). Our recent study(12) also suggested that genistein might be a promising chemopreventive agent due to its capability to modulate cellular detoxification enzyme(s). Daidzein, the other main isoflavone present in soybean, has also been reported to have association with inhibition of human breast cancer cells and retain antiestrogenic activity(3, 13). Identification and quantitation of isoflavones have been performed by several methods including high performance liquid chromatography(HPLC)(6,7,14,15), gas-liquid chromatography(16), isotope dilution GC-MS(17), and thin layer chromatography(18). The assay procedure which was developed by Wang et al.(14) and employing HPLC equipped with C_{18} column looked feasible for large numbers of samples in a regular laboratory.

Isoflavone content, an important determinant of soybean quality, is believed to be variable during maturation of soybean seed. Furthermore Korean people consume soybean at immature stages as well as mature seed. This study has been conducted to determine the change in isoflavone content during soybean seed development.

MATERIALS AND METHODS

Materials

Soybean(*Sinpaldal* var.) with relatively high isoflavone content(19) was sown on the 24th May, 1996 in the private farmland of Kimhae, Kyongnam province, and soybean samples were collected at 13, 14, 15, 17, 19, and 21st weeks after sowing. HPLC-grade solvents were purchased from Merck(Rathway, NJ, USA). Genistein, glycitein and daidzein standards were purchased from Sigma(St. Louis, MO, USA), Plantech(Reading, UK) and Indofine Chemical (Somerville, NJ, USA), respectively. Other reagent grade chemicals were obtained from Sigma. HPLC column(3.9 mm×30cm length), μ-Bondapak C_{18}(10μm), was from Waters Associates(Milford, MA, USA).

Sample preparation

Soybean was powdered using Cemotec mill(Tecator, Sweden). The portion of soybean flour was allotted for

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the proximate analysis. One gram of dry ground sample suspended in 3ml 1M HCl was heated at 98~100°C for 1.5hr in heating block(Thermolyne, Dubuque, USA). At the end of digestion, 4 volumes of methanol was added to achieve the complete solvation of isoflavones. The mixture was settled for a few minutes, and the supernatant was filtered through Corning syringe filter(nylon, ⌀0.2μm) and used for HPLC analysis(14,19).

Proximate analysis of soybean flour

Moisture, protein, fat, and ash were analyzed by A.O.A.C. methods(20).

Quantitation of isoflavones by HPLC

Analysis of isoflavones was performed according to the method described by Wang et al.(14) with a slight modification(19). The 20μl filtrate was injected to HPLC equipped with μBondapak C_{18} column after the system had been equilibrated at ambient temperature and stabilized the UV detector with mobile phase(methanol-1mM ammonium acetate, 6 : 4) at a flow rate of 1ml/min for 30min. Eluent was detected at 254nm and chromatogram was recorded for 20min. Isoflavones were identified by retention time and internal addition of standards, and their contents and recoveries were calculated by comparing peak areas with those of standards subjected to same treatments.

RESULTS AND DISCUSSION

Soybean variety(*Sinpaldal*) with relatively high isoflavone content was grown at Kimhae in 1996 and soybean samples were collected between 13th(May 24th) and 21st weeks(Oct. 20th) after sowing. Climatic data for the period of May to October 1996 in cultivated field are summarized in Table 1 as growth conditions including temperature and precipitation were reported to have a great effect on the isoflavone content of mature soybean seeds(7,21). For instance, the isoflavone content of soybean grown at high temperature was known to be significantly lowered. Protein content remained relatively constant during the rest of maturation period after reaching maximal level at 13th week while fat content was gradually increased until 17th week of sowing(Table 2). Moisture content started to drop rapidly from 17th week after sowing seeds, reaching 7.9 percent by the 21st week.

Free genistein and daidzein contents kept increasing upto 17th week after sowing the seed and then declined rapidly until the final harvest date while glycitein reached the maximum at 14th week and thereafter plunged to a negligible level(Table 3). Total isoflavones showed the stepwise increasing pattern with maturation except glycitein of which content tended to decrease from 19th week after sowing seeds. All isoflavone contents showed almost double increase in the period between 13th and 14th weeks after sowing seeds and another significant increase during the last two weeks of cultivation. Total genistein, daidzein and glycitein contents were 699, 795, 98mg/kg, respectively, in the final sample collected at 21st week after sowing soybeans. In particular, the amounts of free genistein and its conjugated derivatives were estimated to be 16 and 683mg/kg, respectively. The mature soybean (*Sinpaldal* var.) grown in 1994 in Suwon, Korea previously showed significantly higher levels of isoflavones than the same variety investigated in this study(19). Thus the report that isoflavone content was influenced by the location as well as weather was confirmed in this study. Tsukamoto et al.(21) reported a wide variation in total isoflavone contents depending on varieties, sowing dates,

Table 1. Climatic data at Miryang for the period May-October 1996[1)]

Month	Temperatures(°C) Mean	Max	Min	Sunshine duration (hr/month)	Precipitation (mm/month)
May	17.7	31.5	5.7	229.8	68.2
June	21.5	32.6	12.1	100.6	299.6
July	24.8	36.2	16.4	194.0	134.0
Aug	26.3	36.0	17.2	207.0	58.0
Sept	21.1	31.0	10.6	203.4	40.5
Oct	15.1	27.9	0.9	215.5	26.5

[1)]Data from Annual Climatological Report(1997)

Table 2. Relative percentages of some chemical components in soybean at different development stages

Weeks after sowing	Moisture	Protein	Fat	Ash	Carbohydrate[1)]
13	67.8	14.8(46.0)[2)]	2.2(6.8)	2.2(6.8)	13.0(40.4)
14	67.8	11.8(36.6)	3.2(9.9)	1.9(5.9)	15.3(47.5)
15	66.6	12.7(38.0)	4.0(12.0)	2.1(6.3)	14.6(43.7)
17	58.1	16.5(39.4)	7.5(17.9)	2.6(6.2)	15.3(36.5)
19	25.0	30.1(40.1)	10.3(13.7)	5.4(7.2)	29.2(38.9)
21	7.9	37.6(40.8)	14.3(15.5)	5.8(6.3)	34.4(37.4)

[1)]Percentage of carbohydrate was calculated by subtracting all determined components from 100

[2)]Values in parentheses represent relative percentages expressed as a dry basis

Table 3. **Total and free isoflavone contents of soybean at different development stages** (mg/kg dry sample)

	Weeks after sowing					
Isoflavone	13	14	15	17	19	21
Daidzein	186(115)[1)]	370(124)	335(168)	422(193)	496(47)	795(28)
Glycitein	109(22)	193(71)	165(0)	181(17)	187(9)	98(8)
Genistein	81(37)	155(71)	260(69)	309(95)	365(13)	699(16)
Total	376(174)	718(266)	760(237)	912(305)	1,048(69)	1,592(52)

[1)]Values in parentheses represent free isoflavone contents

weather and locations. For instance, total isoflavone content of the same variety was 5.8 times different in response to different sowing dates, with 246 *versus* 1,423 mg/kg. This difference was postulated to be associated with weather conditions such as temperatures during the maturation of soybean seed.

Naturally occurring soybean isoflavones exist mainly in conjugated forms, e.g. glucosides, malonyl and acetyl derivatives(3). While analysis of both the conjugated forms and the aglycones in a single chromatographic run has advantage in terms that it provides the relative amount of each derivative, and that the determination of total isoflavones before and after the acid-hydrolysis of the soybean sample is simple and highly reproducible(14). Furthermore, conjugated isoflavones are believed to be subjected to extensive digestion process in human gastrointestinal tract and absorbed after being converted into the free form which exerts biological effect such as the chemoprevention of hormone-related carcinomas, osteoporosis, and heart diseases(22) According to our data, mature soybeans are expected to have maximum beneficial effect as they contained the highest amount of isoflavones, in particular, genistein. Immature soybean which are largely consumed by Korean people may have the limited advantage due to the relatively low content of isoflavones.

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