Biological Effects of Black Colored Soybean

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ABSTRACT

It has been believed that supplementation of human diets with soybean products markedly reduces human cancer mortality rates. Specifically the black colored soybean has been considered as having a medicinal effect since ancient times in Korea. Six genotypes of black colored soybean and a normal variety were investigated for their biological effect. While the isoflavone content of black colored soybean was higher than the normal soybean, saponin content of the seeds was not different among varieties. In the case of antioxidant activity, black colored soybean showed over 3 times increase in the active effect than the control variety. When feeding soybean extract, a general change of alcohol resolving effect, such as alcohol dehydrogenase activity, was observed in the blood and liver of rats.

Key Words : Black colored soybean, antioxidant activity, anticarcinogen, isoflavone, saponin

INTRODUCTION

Soybean seeds are one of the most important sources of protein and oil in the world. Recently, emphasis has been placed on the chemical composition of soybean seeds for processed soybean foods. Improvement of soybean components has been expected to improve food-processing quality for processed soybean products such as soymilk and various edible ingredients as well as for the traditional soyfoods.

There is much evidence suggesting that compounds present in soybean can prevent cancer in many different organ systems. Soybean has potential roles in the prevention and treatment of chronic diseases, most notably cancer, osteoporosis, and heart disease. In Korea, black colored soybean has been considered to play some important biological activity from ancient times. However there is no evidence to approve the effects of black colored soybean. Only a few reports about the physiological activity of anthocyanin pigments have been reported in recent years.

In this study soyasaponin and isoflavone that are considered to have effects as anticarcinogens were analyzed, also antioxidant and alcohol resolving activity were investigated. On the basis of the results obtained it may be expected to contribute to the improvement of research on biological activity of black colored soybean.

MATERIALS AND METHODS

Soyasaponin analysis

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Black colored soybeans (5 genotypes) and Hwangkeumkong (normal variety) were used for this experiment. Soyasaponin constituent was analyzed according to the general methods described by Tsukamoto (Tsukamoto *et al.*, 1995). The soybean flour was ground into a fine powder with a mill and was extracted at room temperature with 70% EtOH containing 0.1% AcOH. The extract was used directly for HPLC analysis on an ODS column. The group A and B saponins were separated with MeOH-H_2O-AcOH (700:299:1) whereas the DDMP saponins were separated with MeCN-H_2O-AcOH (420:579:1), and that time solvent flow rate was 1.0 ml/min. UV absorption was measured at 205 nm for the group A and B saponins, and at 292 nm for DDMP saponins. The standard, which was prepared according to Tsukamoto's assay, curves were obtained by plotting the soyasaponin concentration as a function peak area obtained from HPLC.

Isoflavone analysis

The quantitative analysis of isoflavone contents was done according to the methods of Kudou (Kudou *et al.*, 1991) and Kitamura (Kitamura *et al.*,1991). Soybean flour was extracted with 70% EtOH containing 0.1% acetic acid for 24 hours at room temperature. After centrifugation of the extract, the supernatant was used directly for the HPLC analysis with ODS commercially packed columns. A 0.1% acetic acid solvent system that contained a linear gradient of acetonitrile that ran from 15% to 40% during 25 minutes was used. The solvent flow rate was 1.0 ml/min, and the UV absorption was measured at 260 nm. Purified soybean isoflavones obtained by Kudou's method were used as standards.

Antioxidant activity

The soybean flour was treated with four different processing methods, soaking in MeOH, stewing, boiling, and roasting. And that time each sample was extracted in MeOH 3 times. After DPPH (1,1-diphenyl-2-picrylhydrazyl) treatment (2×10^{-4} M, 30 minutes), optical density was measured by spectrophotometer at 517 nm and free radical scavenging effect of each sample was indicated as EC_{50} (μg/ml) value.

Alcohol resolving activity

Soybean flours were extracted in EtOH (at room temp., over 2 times every 48hrs), after that evaporated 35 ± 2℃. The extract was supplied to rat (Spraque Dawly, male, 250-300 g weight), and 30 minutes later EtOH was fed also. Subsequently the rat's blood was collected from a vein after 4 hours from alcohol treatment and simultaneously EtOH content in serum was checked. Alcohol dehydrogenase activity in liver was also analyzed.

RESULTS AND DISCUSSION

Soybean saponins, especially DDMP conjugated saponins, have been considered as major active components contributing to the cholesterol-lowering effect of soybeans and inhibiting tumor development. Five varieties of black colored soybeans and Hwangkeumkong (the most popular variety in Korea) were examined for the soyasaponin constituents (Table 1).

The DDMP saponin in Hwangkeumkong was 161.4 mg/100g and black colored soybeans were on average 157.9 mg/100g with a range of 67.2~233.5 mg/100g. Among black colored soybeans, Dawonkong and Miryang # 70 showed 233.5 and 211.8 mg/100g, respectively. From these results, it can be seen that there is no relationship between soybean seed coat colors and soyasaponin contents, it depends more on the variety.

Soybean isoflavone has been considered for reducing the risk of various cancers. Isoflavones exhibit a multitude of medicinal effects that influence cell growth

Table 1. Mean value of soyasaponin contents in black colored soybean

Cultivar	Saponin content (mg/100g)					Total
	Aa	Ab	Ba	Bb	DDMP	
Hwangkeum-kong	56.4	12.9	3.5	6.3	161.4	240.5
Kyungdong #3	8.2	1.4	5.2	53.2	120.6	188.6
Suwon #174	41.3	7.6	-	47.8	156.4	253.1
Miryang #70	29.9	2.7	6.5	66.3	211.8	317.2
Keumjeong-kong #1	28.5	5.3	-	32.8	67.2	133.8
Dawonkong	59.0	4.9	8.3	36.2	233.5	341.9

Table 2. Content of isoflavone compounds in soybean seed (μg/g)

Cultivars	MGe	MD	MGl	Getin	Dzin	Gltin	Dzein	Getein	Gltein	AGe	AD	AGl
Hwangkeum-kong	845.0	384.0	52.8	135.0	67.5	22.2	11.0	4.5	4.25	5.5	3.25	-
Keumjeong-kong# 1	430.0	327.0	59.0	88.5	76.5	25.8	3.0	6.0	30.3	-	2.7	-
Yuseong jaerae	1271.7	826.8	48.5	227.5	137.0	20.3	2.2	7.0	2.7	-	0.17	-
Suwon# 174	1240.0	728.3	33.8	240.8	131.0	16.0	5.2	6.5	2.65	-	0.17	-

* MGe : Malonyl genistin; MD : Malonyl daidzin; MGl : Malonyl glycistin; Getin : genistin; Dzin : Daidzin; Gltin : glycitin; Dzein : Daidzein; Getein : genistein; Gltein : glycitein; AGe : Acetyl genistin; AD : Acetyl daidzin; AGl : Acetyl genistin

Table 3. Antioxidant effect according to four treatmnts in blackcolored soyban unit : EC_{50}(μg/ml)

Cultivar	Soaking in MeOH	Boiling	Stewing	Roasting
Hwangkumkong	over 400	321.8	339.8	over 400
Keomjeongkong #1	174.3	163.2	97.3	102.3
Miryang #70	121.3	158.3	97.8	99.8
Kyungdong #3	124.5	102.8	78.3	89.7
Dawonkong	89.3	68.2	71.3	88.7

and regulation, which may have potential value in the prevention and treatment of cancer. Isoflavone contents of black colored soybean cultivars grown at Suwon were determined by HPLC analysis (Table 2).

Genistin, daidzin, glycitin and their aglycone (genistein, daidzein, glycitein) are the principal isoflavones found in soybean. Total isoflavone aglycone contents of 4 cultivars grown at Suwon varied from 11.9 to 39.3 μg per 1g seed meal. Malonyl and acetyl forms have also been detected but they are thermally unstable and are usually transformed during the processing in glucoside form. The malonylated isoflavone glycosides, which comprised nearly 80% of the total isoflavone contents were the major isoflavone constituent in soybean seed whereas acetylform were detected as a minor group. Soybean grown at Suwon shows a general decrease for contents of all isoflavone components than that of those grown at Chulwon (data not shown). It may be concluded that the climatic

Table 4. Dietary soybean extract effect on alcohol resolving activity in rats

Cultivars		Conc. of ethanol (mM)	Alcohol dehydrogenase activity (nMoles/min/mg prot)
Hwangkeumkong	Control	13.7±1.3	9.3±0.9
	Soy Extract	9.9±1.3**	11.2±0.2*
Keumjeongkong #1	Control	10.6±1.8	23.6±1.8
	Soy Extract	8.7±0.6*	29.5±1.9**
Miryang #70	Control	18.4±3.5	24.6±2.2
	Soy Extract	13.8±4.4**	29.5±3.2**
Kyungdong #3	Control	16.8±1.8	19.5±1.4
	Soy Extract	11.7±0.9**	28.6±1.8**
Dawonkong	Control	16.7±1.1	20.5±0.2
	Soy Extract	13.7±0.9*	24.6±1.8**

1) * and **, significant at 5 and 1 %, respectively

conditions could influence the variation in soybean isoflavone contents.

Antioxidant activity of black colored soybean was analyzed with 4 different treatments (Table 3).

EC_{50} value in Hwangkeumkong, the normal variety with yellow seed coat color, was over 3 times higher than black colored soybean's, which means black colored soybean have high active antioxidant effect than the normal variety. Among 4 different processing methods, stewing gave rise to high antioxidant activity, as we expected. There are so many soybean products made from stewing in Korea, especially Dawonkong and Kyungdong #3 that are small soybeans (10 g<100-seeds weight) with high activity.

After alcohol treatment, one group of male rats (Sprague-Dawley) was fed 400 mg soybean extract/kg diet and alcohol-resolving effect was investigated (Table 4). Concentration of ethanol in the rat's blood was decreased when they were fed a soybean extract, but the amount of the decrease was similar among varieties. Alcohol dehydrogenase activity in the liver of rats was generally increased. This showed that black colored soybean did not show any difference in alcohol resolving activity as compared with the normal soybean variety.

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