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Antioxidant Potentials and Quantification of Flavonoids in Mung Bean (*Vigna radiata* L.) Seeds

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ABSTRACT

Mung bean (*Vigna radiata* L.) is an increasingly important human food source, as well as a new functional agent, mainly due to its potent antioxidant activity. This study was conducted to determine antioxidant activity of fractions from mung bean seeds by measuring DPPH radical scavenging activity and to quantify the flavonoids by means of HPLC analysis. Vitexin and isovitexin were present in both ethanol and water extracts in highest amount. Flavonoids, vitexin and isovitexin were quantified from 195 germplasms of mung beans and their concentrations varied by 4.7 fold. Especially, the breeding line KM99004-4B-2 (Suwon28/KM94004), which has grown in Jeollanamdo Agricultural Research and Extension Services, showed the highest amount (15.88 mg/g) of total flavonoids. The vitexin portion was averaged 70.73±1.38%. High positive correlation (r=0.96*) between vitexin and isovitexin contents showed. However, the flavonoid content showed very low correlation with the 24 growth and ecological characteristics. Seed coats of mung beans had the highest flavonoid amount, showing 50~70 times more than cotyledons. Flavonoid contents in the seed, the cotyledon, and the seed coat were decreased as the seed imbibition time increased.**

Key words : Antioxidant activity, Isovitexin, Seed imbibition, *Vigna radiata*, Vitexin.

INTRODUCTION

Mung bean (*Vigna radiata* L.) has been commonly used as a pharmaceutical or a cosmetic medicinal material. According to the previous report, the cosmetic called Ock-Yong-Seo-Si-San from mung bean seeds has been traditionally used (Huh, 1966). Mung beans also have been used as a therapeutic measure for detoxifying agricultural chemical poisoning, lead poisoning, mumps, and burns (Kim *et al.*, 1997). The ethanol extract or water extract of mung beans are known to contain vitexin and isovitexin. Since vitexin and isovitexin possess antioxidant and topical anti-inflammatory activities, they are currently being added to cosmetics (Jeong *et al.*, 1998; Kim *et al.*, 1998). So far several cultivars of mung beans have been bred in Korea. Of them, cultivar "Eowool" with high synchronized maturity, "Samgang" which has the torn-

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edges leaves and good genetic properties, and "Jangan" which is weevil resistant, have been developed as popular cultivars. The main goal of breeding programme is to select excellent cultivars with synchronized maturity and seed yield.

Phenolic compounds, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and *tert*-butylhydroquinone (TBHQ), have been widely used as synthetic antioxidants in food lipid. Although those antioxidants are considered as safe natural antioxidants, they do not always provide effective protection against in vitro oxidation (Frankle, 1980). Therefore, research on other natural antioxidants has gained momentum as they are considered, rightly or wrongly, to pose no health risk to consumers (Wanasundara and Shahidi, 1994; Wanasundara *et al.*, 1997). Naturally-occurring antioxidative components in foods or plants include flavonoids, phenolic acids, lignan precursors, terpenes, mixed tocopherols, phospholipids, polyfunctional organic acids and also plant extracts such as those of rosemary and sage (Schuler, 1990; Wanasundara *et al.*, 1997).

Initial water uptake by seeds is accompanied by the release of a large volume of gas and by a rapid leakage of biologically active substances. Such factors as the length of soaking duration, water temperature, aeration during soaking, and amount of water must be considered in measuring seed exudation. Larson (1968) reported absorption of water by seeds with seed coat was different from that by seeds without seed coats. Removal of the seed coat allowed rapid imbibition resulting in seed injury presumably by the loss of solute that included monosaccharides, disaccharides, amino acids, and other nitrogen containing compounds.

Most of previous researches have been focused on mainly primary metabolites such as proteins, carbohydrates, fatty acids, etc. (Koh *et al.*, 1997; Lee *et al.*, 1997). A few studies on other secondary metabolites have been done. Furthermore, no studies have been made from an agronomic approach through breeding program by selecting cultivars that contain higher amount of flavonoids. Thus, in this study, we determined antioxidant potentials and quantified flavonoids in mung bean (*Vigna radiata* L.) seeds through measurement of DPPH radical scavenging activity and quantified vitexin and isovitexin by HPLC, and we examined change of the flavonoid content in the mung bean according to the immersion period. The research would be useful to seek genetic resources that contain a great quantity of flavonoids for breeding program.

MATERIALS AND METHODS

Antioxidant activity of flavonoid compounds

Mung bean (*Vigna radiata* L. cv. "Eowool") seeds were harvested from a field at the Jeollanamdo Articultural Research and Extension Services, Naju, Korea, in 2002, oven-dried at 60℃ for 48 h and finely ground. The ground grains (1kg) were extracted by boiling under reflux with 80% methanol for 12 h. The extract was filtered through No.5 paper (Whatman, Clifton, NJ, USA) to remove the fiber debris. The extract was defatted with methylene chloride, and fractioned with ethyl acetate. The filtrate was evaporated to dryness under vacuum at 40℃ using a rotary evaporator (Eyela, Tokyo, Japan). The crude extract was chromatographed through Sephadex LH-20 column eluted with methanol.

In order to measure antioxidant activity, the DPPH (1,1-diphenyl -2-picrylhydrazyl) free radical scavenging activity method was carried out according to the procedure described by Blosi (1958). This test was carried out in 96-well microtitre plates. Fifty milliliters of a 0.02% DPPH solution in EtOH were added to a solution of the compound to be tested in EtOH (200 ml). Absorbance at 517 nm was determined after 30 min (Cao, 1996; Heilmann *et al.*, 2000; Tagashira and

Ohtake, 1998). The radical scavenging activity was obtained from the following equation: Radical scavenging activity (%) = {(OD control - OD sample) / OD control} × 100. As a result of the DPPH test, higher antioxidant activity was found from two fractions. By means of HPLC analysis (Table 1), flavonol glycosides including vitexin and isovitexin were identified (Jeong *et al.*, 1998). However, no antioxidant activity in the methanol extract was observed.

Variation in flavonoid contents from 195 germplasms of mung bean

One hundred ninety five mung bean germplasms including 6 cultivars, 26 breeding lines, and 163 land races were grown at a field of Jeollanamdo Agricultural Research and Extension Services, Naju, Korea, in 2002. Taxonomical identifications were made by measurement of morphological characteristics such as seed coat color, pod length, seed weight and other key characteristics. Standards of vitexin and isovitexin were provided by Kangwon National University, Chunchon, Korea. Preparation of extract was shown in Fig. 1. HPLC used was a Shimadzu system equipped with model LC-10AD pump, SCL-10A controller, SPD-M10A diode array detector, and CLASS-LC10 software. Conditions for analysis were listed in Table 1. Two flavonoid compounds were separated on an HPLC column with different retention time (Fig. 2.).

Table 1. Composition of the ethanol extract of mung bean, as analyzed on an ODS column, with column conditions as listed

Flavonoids	Ret. time	Conditions
Vitexin	6.5 min	Mobile phase : MeOH:H_2O:EtOAc=34.6:60.0:5.4
		Flow rate : 1㎖/min
		Detector : photo diode array 254㎚
Isovitexin	7.5 min	Column : PHENOMENEX 00G-4337-E0, 250 × 4.60㎚, 4㎛

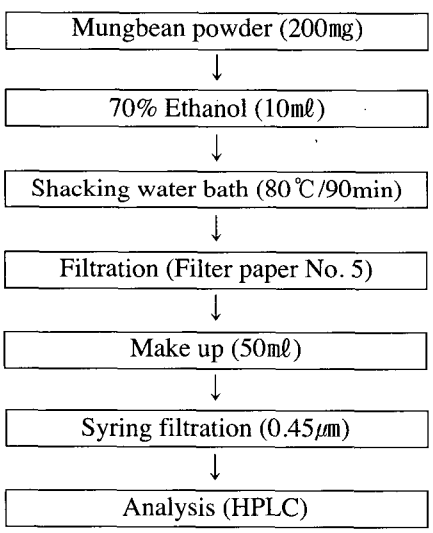

Fig. 1. Extraction method of flavonoids from Mungbean.

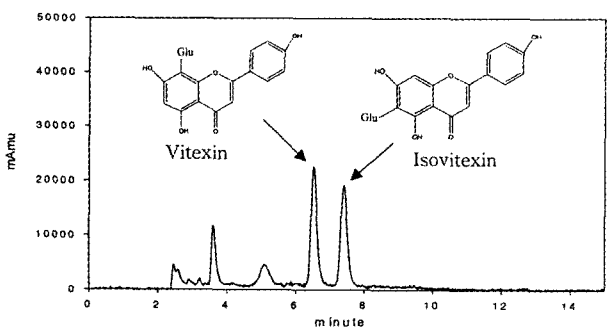

Fig. 2. The HPLC chromatogram of vitexine and isovitexine from the mung bean seed.

Exudation of flavonoid compounds from seeds during imbibition

In order to investigate the change in content of vitexin and isovitexin during imbibition, seeds of cultivars "Eowool" and "Samgang" were imbibed. The imbibed seed coats and their cotyledons were separately collected once a day for 4 days and used for flavonoid analysis. Conditions for HPLC analysis were same to the previous work.

RESULTS AND DISCUSSION

Antioxidant activity of flavonoid compounds

DPPH (1,1-diphenyl-2-picryl hydrazyl) radical scavenging activity was 25.8% and 22.6% for vitexin and isovitexin, respectively, although the activity was less than a synthetic antioxidant BHT (69.4%) (Table 2). The purpose of this experiment was to determine antioxidant potential of the methanol extract. However, no other compounds were found except for vitexin and isovitexin that previously identified (vitexin 5.3 ㎎/g, isovitexin 2.8 ㎎/g) (Table 3). Biological activities of vitexin and isovitexin have been reported by several scientists. Vitexin showed antibacterial effects against Enterobactor cloaceae, E. aerogenes and Pseudomonas aeruginosa (Adriana *et al.*, 1999). The estrogenic activity of vitexin was assayed by employing a reconstituted estrogen transcription unit in *Saccharomyces cerevisiae* transformed with both a human estrogen receptor expression plasmid and a reporter plasmid (Lee *et al.*, 1998). The active components were found to be flavonoid substances, and one of them exhibiting antioxidant activity as strong as α-tocopherol was identified as isovitexin, a C-glycosyl flavonoid in methanol extract from rice seed (Narasimhan *et al.*, 1989). Vitexin and isovitexin have been isolated and identified in buckwheat grain (Dorota and Oleszek, 1999)

Variation in flavonoid contents in 195 germplasms of mung bean

This experiment was designed to provide fundamental genetic information on germplasms for breeding program. The results of quantitative analysis on 195 resources are shown in Table 3. The vitexin content in resource varies from 2.39 ㎎/g to 11.48 ㎎

Table 2. Radical scavenging activity of samples examined by DPPH method

Sample	Absorbance (OD_{517nm})	Radical scavenging activity
		%
Control	0.490±0.005	0.0±0.00
Vitexin	0.364±0.009	25.8±1.61
Isovitexin	0.379±0.004	22.6±1.43
BHT	0.150±0.019	69.4±3.62

Table 3. Distribution of vitexin and isovitexin content in seeds from 195 mungbean germplsms

Flavonoids	Content (㎎/g)						
	~2.00	2.01~4.00	4.01~6.00	6.01~8.00	8.01~10.00	10.01~	Mean±S.D.
	Distribution (%)						
Vitexin	0	17.4	37.5	41.5	3.1	0.5	5.63±1.50
Isovitexin	26.7	72.8	0.5	0	0	0	2.33±0.63

/g, showing 4.8 fold range in its content in 195 germplasms. The isovitexin content in germplasms varied from 0.97㎎/g to 4.40㎎/g, which showed 4.5 fold range in content. In particular, the breeding line KM99004-4B-2 (Suwon28/KM94004, '03 yield trial No. 6) that has grown in Jeollanamdo Agricultural Research and Extension Services (Naju, Korea) had flavonoids as the highest amount (15.88㎎/g). The results suggest that the breeding line has a significant value as a cultivar or a parent for breeding program. Content of isovitexin showed similar tendency as vitexin according to the breeding line. The vitexin content was 70.73±1.38% (67.5~74.0%) of total flovonoids contained in mungbean seeds. No significant difference in content ratio of vitexin among 195 germplsms was observed. Content of vitexin was highly positively correlated with isovitexin (Fig. 3).

However, correlation between flavonoid contents in mung bean germplasms and physiologic and ecological characteristics was very low, indicating that production of flavonoids was not affected by 24 physio-ecological characteristics (Table 4). The result showed that the flavonoids present in mung beans can not be based on certain physio-ecological characteristics. Since no related studies have been done at this aspects, further detail studies are required to prove the assumption.

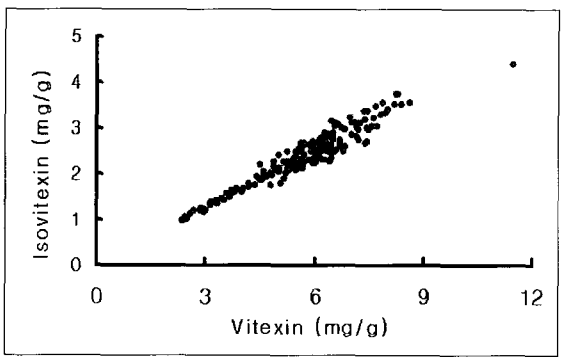

Fig. 3. Correlation between vitexin and isovitexin contents contained in the mung bean (r=0.96***).

Exudation of flavonoid compounds from seeds during imbibition

To determine the changes of content of vitexin and isovitexin according to the imbibition period of the mung bean, seeds of "Eowool" and "Samgang" mung bean cultivars were imbibed and taken out for HPLC analysis after imbibition. The results showed that the changes in weights of the seed coat, seed and cotyledon of mung bean were reduced as the immersion period increased (Fig. 4). Seeds of two mung bean cultivars "Eowool" and "Samgang" were imbibed, separated into seed coat and cotyledon, and collected once a day over 4 days. The contents of flavonoids present in the seed, the cotyledon, and the seed coat were gradually reduced with the increasing of the imbibition period (Fig. 5). The results indicate that the flavonoid content was decreased due to various physiological metabolism or leaching during imbibition at germination. On the other hand, the contents of vitexin and isovitexin in seed coat were around 50-70 times more than those in cotyledon. This results showed that imbibing seeds have more flavonoids than seedling sprouts for mung bean utilization as a food or nutrient agent.

Initial water uptake by seeds is accompanied by the release of a large volume of gas and by a rapid leakage of substances, e.g. sugars, organic acids and amino acids. Such factors as the length of soaking duration, water temperature, aeration during soaking, and amount of water must be considered in measuring seed exudation. Larson (1968) reported that water absorption by seeds with seed coat was slower than that by seeds without seed coats. Removal of the seed coat allowed rapid imbibition resulting in seed injury presumably because of the loss of solute that included monosaccharides, disaccharides, amino acids, and other nitrogen containing compounds.

Table 4. Correlation of the physiologic and ecological characteristics and the flavonoid content of mung bean

Characteristics	Vitexin (A)	Isovitexin (B)	A+B
Growth habit	0.030	-0.059	0.002
Hypocotyl color	-0.037	-0.016	-0.031
Leaf color	-0.037	-0.016	-0.031
Leaf size	0.011	-0.055	-0.009
Petiole color	0.030	-0.059	0.002
Pubescence density	-0.102	-0.113	-0.106
Stem length	-0.108	-0.098	-0.106
Stem diameter	0.060	0.176	0.096
Nodes of main stem	-0.001	0.023	0.007
Branch number	0.048	0.131	0.074
Days to flowering	-0.101	-0.121	-0.115
Days to maturation	-0.130	-0.199	-0.153
Pods per plant	-0.106	0.000	-0.074
Forming node of the primary pod	0.091	0.082	0.089
Pod color at maturity	-0.018	0.006	-0.011
Seed per pod	0.085	0.013	0.064
Pod length	-0.097	-0.058	-0.086
Pod curve	-0.010	-0.048	-0.022
Shattering property	0.184	0.197	0.189
Seed coat color	-0.070	-0.063	-0.069
Luster on seed surface	-0.050	-0.087	-0.062
Seed weight	-0.118	-0.093	-0.111
Seed yield	-0.079	-0.038	-0.067
Lodging	-0.057	-0.040	-0.052

*Note : No significant difference in contents vitexin and isovitexin among 195 germplsms was observed. Correlation between flavonoid contents in mung bean germplasms and physiologic and ecological characteristics was very low, indicating that production of flavonoids was not affected by 24 physio-ecological characteristics.

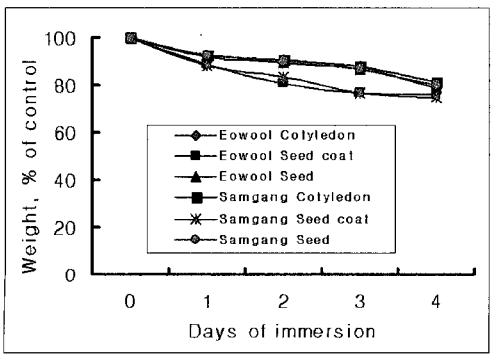

Fig. 4. Changes in the weight of seed, cotyledon, and seed coat of two cultivars according to the immersion period.

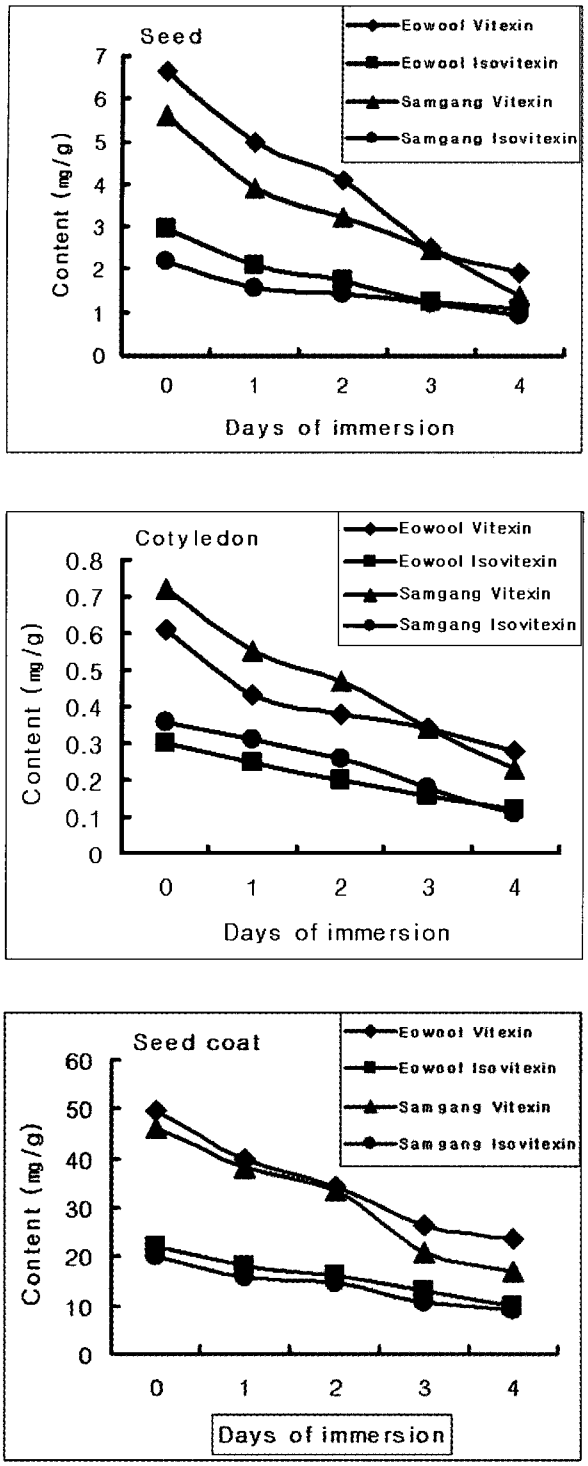

Fig. 5. Changes in the flavonoid content in each part of the mung bean according to the immersion period.

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