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RESEARCH NOTE

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Metabolism of Isoflavone Derivatives During Manufacturing of Traditional *Meju* and *Doenjang*

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Abstract *Meju*, a major ingredient of *doenjang* which is a popular Korean traditional fermented soyfood, was manufactured by fermenting steamed soybeans in natural environment in which steamed soy was exposed to airborne microorganism, in particular, fungi. Total isoflavone content was reduced from 1,849 mg/kg of cooked soy to 816 mg/kg of *meju* at the 90th day of fermentation. Total glycosides and aglycones of isoflavones in *meju* were 1,827 and 22 mg/kg at 0 day and changed into 487 and 329 mg/kg at the 90th day of fermentation, respectively. Meanwhile, the ratio of glycosides to aglycones of isoflavones was not changed during aging of *doenjang* but remained relatively constant with 592 and 644 mg/kg aglycones at the 0 and 160th day, respectively. When cooked soy was fermented with *Aspergillus oryzae* and *Aspergillus flavus*, isoflavone profiles were significantly different from each other while *A. oryzae* caused more extensive metabolism of isoflavones than *A. flavus*.

Key words: *meju, doenjang, koji, miso*, isoflavone, fermentation

Introduction

Soy isoflavones attracted much attention due to their potential to prevent and treat chronic diseases such as osteoporosis, coronary heart disease, postmenopausal complications, and sex-hormone related cancers (1-3). Isoflavones are mostly present in glycoside forms in unprocessed soybean and are metabolized into aglycones during fermentation processes such as preparation of *cheonggukjang*, *doenjang*, *miso*, *tempeh*, and others. Isoflavonoids from legumes were reported to be hydrolyzed by microorganisms in the large intestine prior to absorption. It is expected that alycone forms of isoflavones might be more effectively absorbed than glycosides although recent studies demonstrated that bioavailability of glycosides are as equally efficient as aglycones (4). Therefore, fermentation of soybean will enhance the health promotion function of isoflavones through conversion of glycosides into aglycones.

Soy isoflavones act as weak estrogens or anti-estrogens depending on their concentration in the medium (5). The physiological function of isoflavones appears to be mediated by a variety of mechanisms including estrogenic activity, inhibition of topoisomerase and tyrosine kinase, cell cycle arrest, and so on (6-10). There are 12 chemical forms of isoflavones in soybeans and soy foods. Genistein, daidzein, and glycitein are the aglucones, with 3 possible glucoside forms, a β-glucoside, a 6"-*O*-malonyl-glucoside and a 6"-*O*-acetyl-glucoside (11,12). The concentrations of these forms will vary in soy foods depending upon how they are processed (13). *Doenjang* has been manufactured for centuries at home by traditional methods, in which natural microflora, in particular, storage fungi and *Bacillus subtilis* are used (8). To prepare traditional *doenjang* soybeans are cleaned, soaked in water, and boiled or steamed. These cooked soybeans are crushed and molded as a brick shape (*meju*), dried for 2 days in the air, hung up by rice straw and fermented for 30-90 days. The fermented *meju* is brined and ripened for 2-3 months. When completing *meju* ripening, it is separated into liquid and solid parts. The liquid part is used for making *ganjang* (soy sauce). The crushed solid part is further aged in a separated pottery for more than 2 months (2-6 months) and become *doenjang*. In this study we investigated the change of isoflavone composition during preparation of traditional *meju* and *doenjang* according to the methods used in the 'Sunchang femented soy products valley'.

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Materials and Methods

Samples *Meju* and *doenjang* prepared by traditional methods were obtained from a manufacturer in the 'Sunchang femented soy products valley'. The procedures for manufacturing *meju* and *doenjang* are as follows; soybeans cultivated in the Sunchang area were soaked in water at 20°C for 18-20 hr, and steamed for 30 min at 121°C. The steamed soybeans were formed in blocks of which sizes were 10×8×20 cm, left hung up outdoors for 3 months. Samples were collected at the 0, 10, 20, 40, 90th day of fermentation. *Doenjang* was prepared by crushing *meju* submerged in 26% salt solution (50 kg dry *meju* plus 50 kg salt solution) for 53 days,

sprayed with 1 kg salt per 100 kg swollen form of *meju* (or *doenjang*) on the top, wrapped with vinyl cover, and aged in earthen pot for 4 months in the outdoors. Samples were collected at the 0, 20, 40, 60, 80, 120, 140 160th days and subjected to isoflavone analysis.

Isoflavone standards Isoflavone standards were obtained as follows: genistein, daidzein, and glycitein from Sigma-Aldrich (St Louis, MO, USA), genistin, daidzin, glycitin from Indofine (Hillsborough, NJ, USA), and malonyl genistein, malonyl daidzin, malonyl glycitin, acetyl genistein, acetyl daidzin, acetyl glycitin from LC Labs (Woburn, MA, USA).

Isoflavone analysis Freeze-dried *meju* and *doenjang* was powdered in a Food Mixer (FM-909T, Hanil, Seoul, Korea), and 2 g samples were extracted in 10 mL of acetonitrile, 2 mL of 0.1 N HCl, and 7 mL of water in a 125 mL screw-top Erlenmeyer flask with stirring for 2 hr at room temperature according to Murphy's procedure (14). The residues were dissolved in 80% high-performance liquid chromatography (HPLC) grade methanol. An aliquot was filtered through a 0.45 μm nylon filter (Nunc, Rochester, NY, USA) and analyzed by HPLC. A Jasco chromatograph with a autosampler (model AS 2055), a model PU 1580 dual pump, and a ultra violet (UV)-visible detector (UV-2077) was used to analyze each sample. A Phenomenex Gemini C18 column (5 μm, 150×2.00 mm) was employed for chromatographic separations. A linear gradient composed of A (0.1% phosphoric acid in water) and B (acetonitrile) was used. After injection of a 10 μL sample, the system was increased from 10 to 35% B over 40 min, returned to 10% in 5 min, and maintained at 10% B for another 10 min. The system was recycled to 10% B at the end of 55 min. The flow rate was 0.8 mL/min. The UV absorbance was monitored at 280 nm. UV spectra were recorded and peak areas were integrated using Young-Lin Autochro 2000 software (Young-Lin, Anyang, Korea). Analyses were repeated 3 times and data were expressed as the mean± standard deviation (n=3). To determine the recovery of isoflavones, stock solutions of authentic isoflavone standards and fluorescein were added to samples before isoflavone extraction (14).

Results and Discussion

In this study we investigated changes of isoflavone content during the preparation of *meju* and *doenjang*. As shown in Fig. 1, 12 isoflavone standards could be effectively separated from each other under the analytical conditions used. The total amount of isoflavones in raw soybeans was 3,137± 174 mg/kg, and decreased to 1,849±23 mg/kg after soaking and steaming processes (Table 1). Most of isoflavones in cooked and unfermented soybean existed in glycosides, accounting for 98.8% of total isoflavones. Further reduction in total isoflavone content occurred during 90 days of fermentation period. While isoflavone content was not changed significantly during the first 10 days of fermentation, its content showed dramatic decrease, from 1,849 to 816 mg/kg between the 10th and 90th day from the start of fermentation, suggesting the rapid growth of microorganisms and production of isoflavone-degrading enzymes. While total glycosides decreased from 1,827 to 487 mg/kg during *meju* fermentation, the concentration of aglycones was increased from 22 to 329 mg/kg during the same period. After 90 days of fermentation, the percentage of aglycones to total isoflavones in *meju* was approximately 40%. The conversion of glycosides to aglycones is dependent upon β-glucosidase activity derived from soybean itself or produced by microorganisms. We identified *Aspergillus oryzae* as the major fungus growing in *meju* prepared by traditonal method. The microorganism has been reported to show relatively high β-glucosidase activity, compared to other *koji* starters including *Aspergillus awamori, Rhizopus*

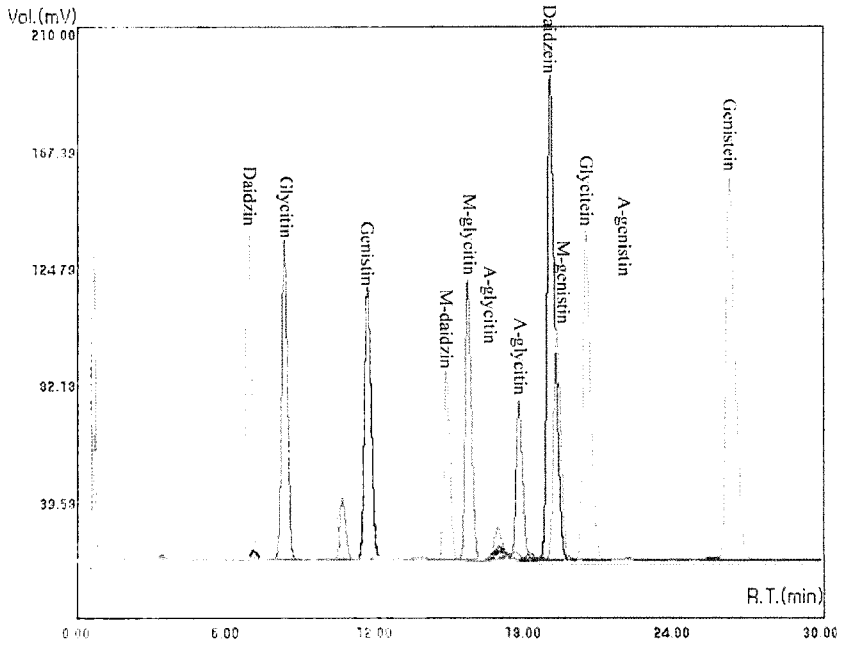

Fig. 1. Chromatogram of 12 standard isoflavones and their glycosides.

Table 1. Change of isoflavone content (μg/g) during *meju* fermentation

Isoflavone	Raw	Steamed	Fermentation period (day)			
			10	20	40	90
Daidzin	98±18	454±3	367±22	355±19	334±8	110±28
Glycitin	94±51	104±16	93±18	77±37	67±23	13±20
Genistin	148±21	913±11	637±62	623±21	488±9	229±33
M-Daidzin	1,080±44	61±3	280±31	165±14	196±6	92±41
M-Glycitin	233±24	1±1	5±9	12±1	3±5	10±9
M-Genistin	1,244±39	74±17	264±10	Tr[1)]	Tr	Tr
A-Daidzin	23±16	55±5	50±8	47±0	46±8	14±1
A-Glycitin	134±19	26±19	82±8	13±2	11±8	6±2
A-Genistin	57±10	139±3	53±22	45±19	35±17	15±24
Total glycosides	**3,111±177**	**1,827±20**	**1,830±125**	**1,337±52**	**1,181±71**	**487±220**
Daidzein	Tr	Tr	Tr	74±3	68±9	152±19
Glycitein	13±7	6±1	Tr	Tr	4±5	7±2
Genistein	12±2	16±1	6±3	35±3	25±3	170±20
Total aglycones	**25±6**	**22±2**	**6±4**	**108±6**	**97±9**	**329±56**
Total isoflavones	**3,137±174**	**1,849±23**	**1,836±123**	**1,445±47**	**1,278±65**	**816±276**

[1)]Trace.

Table 2. Change of isoflavone content (μg/g) during aging of *doenjang*

Isoflavone	Aging period (day)							
	0	20	40	60	80	120	140	160
Daidzin	Tr[1)]	Tr	Tr	Tr	Tr	Tr	Tr	Tr
Glycitin	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr
Genistin	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr
M-Daidzin	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr
M-Glycitin	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr
M-Genistin	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr
A-Daidzin	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr
A-Glycitin	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr
A-Genistin	27±5	36±7	32±5	42±7	41±8	42±17	29±8	16±2
Total glycosides	**27±5**	**36±7**	**32±5**	**42±7**	**41±8**	**42±17**	**29±8**	**16±2**
Daidzein	222±34	246±67	226±23	282±23	271±50	275±47	325±55	273±24
Glycitein	72±14	78±21	72±4	82±5	82±17	71±16	89±22	79±23
Genistein	298±27	366±81	349±42	411±25	361±40	374±36	334±61	292±55
Total aglycones	**592±70**	**689±167**	**646±64**	**775±50**	**714±106**	**719±75**	**749±110**	**644±14**
Total isoflavones	**618±75**	**724±175**	**678±69**	**817±56**	**754±114**	**761±92**	**778±111**	**660±12**

[1)]Trace.

azygosporus (16). In fact, we found that the percentage of aglycones to total isoflavones was 85% in soybean fermented with *A. oryzae* isolated from *meju* made traditionally while it was 20% in soybean treated in a similar way as fermented soy but not inoculated with any microorganism (Table 3).

Though still controversial, the aglycone forms of isoflavones seem to be better bioavailable and faster absorbed than glycoside forms (17). Isoflavone aglycones may be absorbed faster than glucosides because they have greater hydrophobicity and a smaller molecular weight (18) and also because glucosides have less absorbability and must be converted to aglycone forms (19).

A recent study reported that the food matrix is an important factor in the bioavailability of isoflavones (4,20, 21). For instance, consumption of *tempeh* (mainly isoflavone aglycones) resulted in higher serum peak levels of both daidzein and genistein compared with textured vegetable protein (predominantly isoflavone glucosides) (4).

Major isoflavone loss during *doenjang* preparation occurred at the *meju* fermentation step. But no significant change in isoflavone content was observed during aging of *doenjang* probably due to inhibition of microorganism growth under high salt condition. This observation suggests that the development of novel processes to minimize isoflavone loss during *meju* fermentation is of essential

Table 3. Change of isoflavone content during soy fermentation by *Aspergillus* species

Isoflavone	Concentration (μg/g)		
	Control	*A. flavus*	*A. oryzae*
Daidzin	99±44	64±20	82±16
Glycitin	78±18	70±13	70±11
Genistin	13±16	66±24	7±9
M-Daidzin	335±146	184±25	72±16
M-Glycitin	66±2	84±55	11±14
M-Genistin	532±195	91±158	16±28
A-Daidzin	33±38	99±75	6±4
A-Glycitin	135±68	129±45	3±0
A-Genistin	5±9	46±33	5±5
Total glycosides	**1,288±135**	**837±258**	**271±46**
Daidzein	96±61	313±67	478±5
Glycitein	70±12	58±6	191±7
Genistein	174±9	265±14	792±15
Total aglycones	**340±49**	**635±83**	**1,462±18**
Total isoflavones	**1,628±97**	**1,471±207**	**1,733±43**

importance for promoting health benefit of *doenjang* by improving its retention of isoflavones. Furthermore, screening of GRAS-grade microorganisms with high β-glucosidase activity should be preceded for manufacturing high quality *doenjang*. It has also been reported that traditional *doenjang* showed stronger antimutagenic activity than the commercially prepared, *miso*, and *cheonggukjang* (8). Relatively high level of aglycones such as genistein in traditional *doenjang* may explain high antimutagenic and anticancer potential, compared to other soy products such as *cheonggukjang* (22,23). Generally, the fermentation process of soybean increases ratio of aglycone forms of isoflavones to glycosides and thereby enhance the potential of soybean to prevent and treat chronic diseases (8,24,25)

In conclusion, this study demonstrated that extensive isoflavone metabolism occurred during *meju* fermentation but not during aging of *doenjang*, leading to significant decrease of total isoflavones and comprehensive conversion of glycosides into aglycones.

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