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Changes in Isoflavone Content and Mass Balance During Soybean Processing

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Abstract We analyzed the isoflavone content of domestic soybeans during steaming, boiling, fermentation, germinating cultivation, fermentation, and soybean curd production. The isoflavone content of the beans was reduced by steaming and boiling, and overall reductions ranging from 16.0 to 65.0% of initial isoflavone values were detected. After 4 days of germinating cultivation, the total isoflavones of *Eunhakong* increased from 1,341 to 2,017 μg/g and the total isoflavones of *Guinunikong* increased from 1,284 to 1,535 μg/g. The isoflavone content of the vinegar beans produced from *Hwangkeumkong* and *Black* No. 1 increased from 1,877 to 1,956 μg/g, and from 885 to 1,956 μg/g after 8 days of immersion in 4% acetic acid, respectively. During soybean curd production, significant amounts of isoflavones were lost in the whey (30-31%) and soybean curd residue (15-20%). Only 37.4% of the isoflavones present in the original soybeans remained in the soybean curd with the hot extraction method, and 50.7% of them with the cold extraction method. Soybean curd prepared with whole soybean method, however, retained 80.7% of the initial isoflavones.

Keywords: soybean, isoflavone, retention, processing method, soybean curd

Introduction

Soybeans contain not only high quality protein, unsaturated fatty acids, and other nutritive components, but also functional substances with beneficial physiological effects (1, 2). In particular, soy contains several non-nutritional factors, and its value has increased since the discovery that it also contains substances with anticancer activities (3, 4). The primary functional substances in soybeans are dietary fiber, oligosaccharides, isoflavones, phytic acid, protease inhibitors, saponins, proteins and peptides, phytosterols, and phenolic compounds. Based on a large number of studies showing the health benefits of isoflavones, phytic acid, and saponins, isoflavones are considered to be the most beneficial soy components (5, 6). Soybean isoflavones have antioxidant and hormonal properties that may reduce the risk of coronary heart disease, cancer, and osteoporosis, and they have been shown to alleviate hot flashes in menopausal women (7-9). Isoflavones have also been suggested to help prevent many hormone-dependent diseases, including breast and prostate cancers, due to their weak estrogenic activity (10-12).

Several isoflavones have been identified in soybeans and soy foods, including daidzein, glycitein, genistein, and their glucosides. The content and form of the isoflavones present in soy products can vary widely, and they are influenced by the specific manufacturing processes applied (13, 14). Wang and Murphy (15) studied the loss of isoflavones from soybeans during the production of *tempeh*, soybean curd, and soy isolate, and found that 61, 44, and 53% of the total isoflavone contents were lost, respectively, during the manufacturing process.

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Every soy product on the market has different isoflavone content due to differences in processing (16). The loss of isoflavones during processing has been attributed to the hydrolysis of glycosidic conjugates and to the leaching of isoflavones into the discarding water (17-19). Therefore, it is necessary to develop procedures that can preserve the content of effective components in the end products.

In this study, we examined the effects of processing conditions on the retention of isoflavones during preparation of commonly used soy foods in Korea, by analyzing changes in isoflavone content during steaming, boiling, fermentation, germinating cultivation, and soybean curd production.

Materials and Methods

Materials Soybeans (*Glycine max* L.) were purchased from the Moolahnbo Agricultural Association in Cheongju, Korea. Two traditional Korean soybean cultivars, *Black* No. 1 (black soybeans) and *Hwangkeumkong* (yellow soybeans), were used. The materials were stored in desiccators at -20°C until use. The small soybeans for growing soybean sprouts, *Guinunikong* (black beans) and *Eunhakong* (yellow beans), were obtained from the Yeongnam National Crop Experiment Station in Miryang, Korea. Most of the standards, including genistein and daidzein, were purchased from Sigma (St. Louis, MO, USA), while glycitein and other isoflavones were obtained from Fujicco (Tokyo, Japan).

Steaming Twenty g aliquots of *Black* No. 1 and *Hwangkeumkong* were soaked for 12 hr at 4°C. After soaking, the samples were blotted to remove surface moisture and half were steamed for 15, 30, 45, and 60 min at 95°C and atmospheric pressure, while the other half were steamed for 10, 20, and 30 min at 121°C. The steamed soybeans were then lyophilized and ground for HPLC analysis. All

procedures were carried out in triplicate. The moisture content of all of the samples was determined to calculate their isoflavone contents on a dry basis.

Boiling Twenty g portions of each sample type were soaked for 12 hr at 4°C. Raw beans and presoaked beans were then heated separately for 15, 30, 45, and 60 min in boiling water. After boiling, the soybeans were lyophilized and ground for HPLC analysis.

Germinating cultivation One hundred g each of the *Guinunikong* and *Eunhakong* were placed in a cultivation chamber (Dosung Co., Seoul, Korea) at 25°C, and sprout samples were collected every 4 days. The sprouts were then lyophilized and ground for use as HPLC samples.

Preparation of vinegar beans One hundred g portions of each sample type were washed and the moisture was removed from their surfaces. The beans were then divided into airtight containers, covered with 4 or 7% acetic acid, and kept at 25°C. After 2, 4, 6, 8, and 10 days, the soybean samples were collected and soaked in vinegar to remove the residual acetic acid solution from their surfaces. The beans were then lyophilized and ground for use as HPLC samples.

Fermentation of soybeans *Meju* (a fermenting block for making traditional Korean soy sauce or soy paste) and *doenjang* (a traditional Korean fermented soybean paste) were produced according to a traditional method (20). Here, the beans are boiled and ground by a rock into fine bits and formed into a block, which is called *meju*. As a starter culture, soybeans presoaked in water for 24 hr were boiled at 121°C for 60 min. The steamed beans were ground and formed into a block (15×10×20 cm^3), inoculated with *A. oryaze* CF 002P (Chungmu Co., Busan, Korea), fermented at 29°C for 20 days, and then fermented at 20°C for 30 days after molding. To manufacture the *doenjang*, *meju*, salt (sodium chloride), and water were mixed. The mixture was then fermented for 4 months.

Preparation of soybean curd As shown in Fig. 1, soymilk for making soybean curd was prepared from the *Hwangkeumkong* using three different methods: cold extraction, hot extraction, and whole soybean. The soymilk was cooled to 80°C and coagulated using calcium sulfate. After settling for 10 min, the curd was transferred to a cheesecloth-lined wooden box (13×9×7 cm^3) and pressed by a 1 kg weight placed on top for 30 min (21). The soybean curd, soybean curd residue, and soybean curd whey were each lyophilized separately.

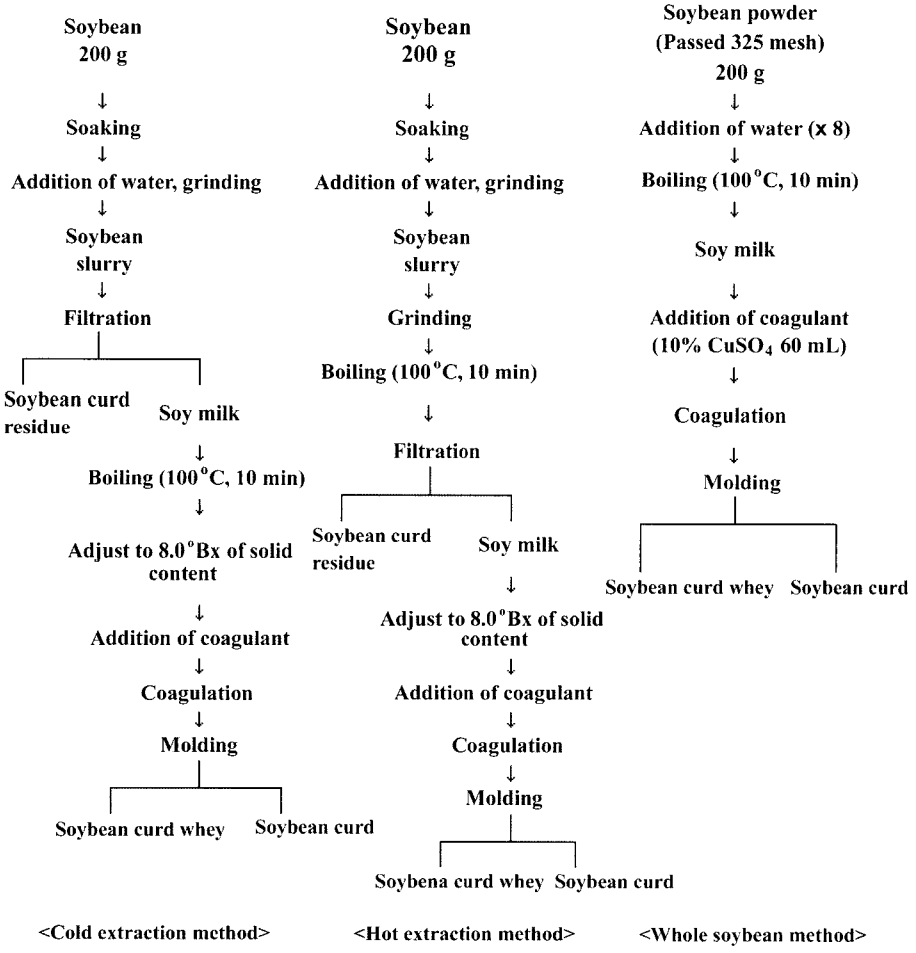

Fig. 1. Various preparation methods of soybean curd.

Extraction and isoflavone analysis The isoflavone contents of the samples were analyzed using the acid hydrolysis method of Wang *et al.* (22). Briefly, 24 mL of 1 M HCl were added to 1-5 g of each ground sample and heated at 95°C in a heating bath. The mixtures were then cooled, and methanol was added to a final volume of 100 mL. The solutions were stirred for 12 hr, centrifuged at 12,000×g for 5 min, and the supernatants were filtered and analyzed by HPLC (Jasco Corperation, Tokyo, Japan) using an ODS AM 303 column (4.3×250 mm; YMC, Milford, MA, USA). A mixture of acetonitrile containing 0.1% acetic acid (solvent A) and water with 0.1% acetic acid (solvent B) was used as the mobile phase. To analyze the isoflavone profile, a linear gradient of 15 to 35% of solvent B in solvent A was applied over 50 min. The flow rate was roughly 1.0 mL/min, the injection volume was fixed at 20 μL, and the wavelength of the UV detector (Jasco Corporation) was set at 254 nm.

The standard isoflavones were dissolved in methanol at 1-100 μg/mL. The percentage of isoflavones retained in the samples was calculated from the initial total isoflavone concentration (μg/ 100 g of dry soybean) and the isoflavone mass after treatment. Because heat alters the distribution of individual isoflavone glucosides, the total isoflavone content was analyzed after conversion of the glucosides to aglycones by acid hydrolysis. Figure 2 shows the HPLC chromatograms of the extracted isoflavones before and after acid hydrolysis. Determining the total isoflavone content after acid hydrolysis allowed us to account for changes in mass balance that may not have been resolved by detecting the distribution of isoflavone isomers.

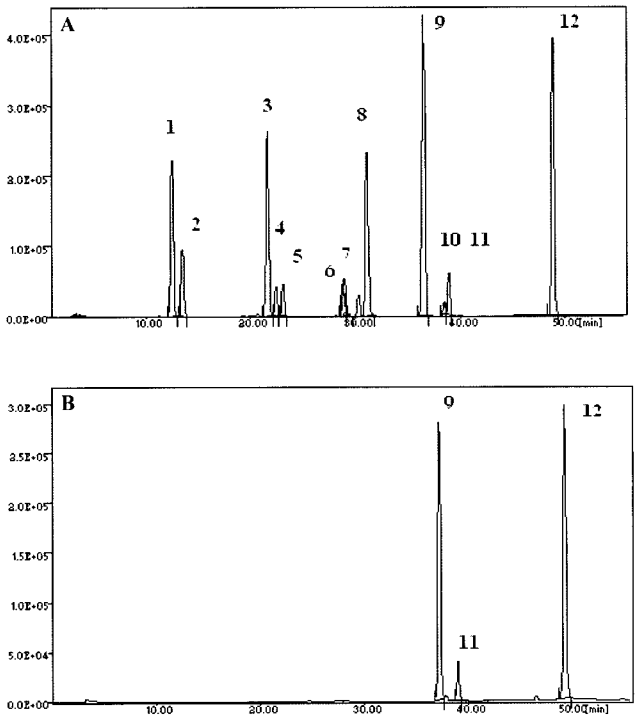

Fig. 2. HPLC chromatograms of isoflavones extracted from soy products before (A) and after (B) acid hydrolysis. 1, daidzein; 2, glycitein; 3, genistin; 4, malonyldaidzin; 5, malonylglycitin; 6, acetyldaidzin; 7, acetylglycitin; 8, malonylgenistin; 9, daidzein; 10, acetylgenistin; 11, glycitein; 12, genistein.

Results and Discussion

Effects of steaming The effects of steaming on the *Black* No. 1 and *Hwangkeumkong* were investigated at and above atmospheric pressure. A treatment at atmospheric pressure for 15 min at 95°C reduced the weight of the soybeans to 82.2 and 78.3% of their initial values, respectively. After 60 min of treatment, the weights were further decreased to 77.0 and 75.7% of their initial values. At 121°C under high pressure (0.15 MPa), the weights were lowered to 85.5 and 80.7% of their initial values after 10 min, and to 77 and 75.7% after 60 min for the *Black No. 1* and *Hwangkeumkong*, respectively. The reductions in weight resulted from the elution of solids during steaming.

As shown in Fig. 3, an overall reduction in isoflavone content by 16.0 to 50.1% was detected in the steamed beans. The isoflavone level dropped sharply during the first 15 min of treatment in all of the samples; however, it was not greatly affected by additional treatment. The effect of the pressurized and atmospheric conditions was mostly related to the weight of the samples, and the effect of the steaming temperature on the isoflavone content was not significant. According to the data, the steam-treated *Black* No. 1 had lower isoflavone content than the steam-treated *Hwangkeumkong*. This result may have been due to differences in structure, or the coat properties of the soybeans. A significant decrease in total isoflavones was not seen over the steaming time.

Effects of boiling Boiling is a common step in soy foods production. Dried and presoaked soybean samples were boiled separately in water as a heat treatment. The overall change in the isoflavone content of the samples was the

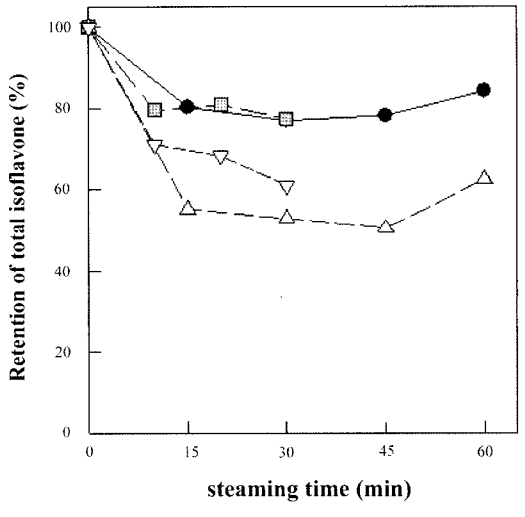

Fig. 3. Changes in the isoflavone content of soybeans during normal steaming (95°C) and pressurized steaming (121°C, 0.15 MPa). ●, Isoflavone content of *Hwangkeumkong* after steaming at 95°C; ■, isoflavone content of *Hwangkeumkong* after steaming at 121°C; ▲, isoflavone content of *Black* No. 1 after steaming at 95 °C; ▼, isoflavone content of *Black* No. 1 after steaming at 121°C.

same as in Fig. 4. The weights of the dried *Black* No. 1 and *Hwangkeumkong* samples were reduced to 92.0 and 90.7% of their initial values, respectively, after treatment at 95°C for 15 min, whereas the weights of the presoaked samples were reduced to 77.4 and 78.3% of their initial values, respectively. Boiling the presoaked *Black* No. 1 and *Hwangkeumkong* samples for more than 60 min lowered their weights to 71.9 and 72.6% of their initial values, respectively, whereas the similarly treated dried soybeans were only reduced to 79.7 and 79.0% of their initial values, respectively. As shown in Fig. 4, as the boiling time increased, the isoflavone content of the samples decreased, and a greater reduction was observed in the presoaked groups. The original isoflavone contents of the *Black* No. 1 and *Hwangkeumkong* samples were 1,876 and 876 μg/g, respectively, but they decreased to 1,238 and 426 μg/g, respectively, following 60 min of boiling after presoaking. The reduction in the amount of isoflavones caused by boiling was much greater than that by steaming, likely due to the increased leaching of solids and isoflavones into the cooking water. Wang and Murphy (15) reported that during the production of *tempeh*, most isoflavones leached out into the cooking water. Processes involving liquids, such as soaking and boiling, have been shown to reduce isoflavone content due to leaching (23).

Effect of germinating cultivation Previous studies have shown that changing the conditions of germination alters soybean composition, including the levels of protein, lipids, and vitamins (24). In this study, as the germination period was increased, the total isoflavone content of the samples increased (Fig. 5). After 4 days of cultivation, the total isoflavone content of the *Eunhakong* sprouts increased from 1,341 to 2,017 μg/g and the total isoflavone content of the *Guinunikong* sprouts increased from 1,284 to 1,535 μg/g. In addition, the levels of daidzein and genistein increased while the level of glycitein remained unchanged. After 4 days of cultivation, the isoflavone level in the harvested soy sprouts was 110% for *Eunhakong* and 97% for *Guinunikong*. It was clearly shown that the germination process retained or slightly increased total isoflavone content in the soybeans. It was reported that total isoflavone content increased by 13% during initial germination, and the aglycone form dominated the increase in isoflavones (25).

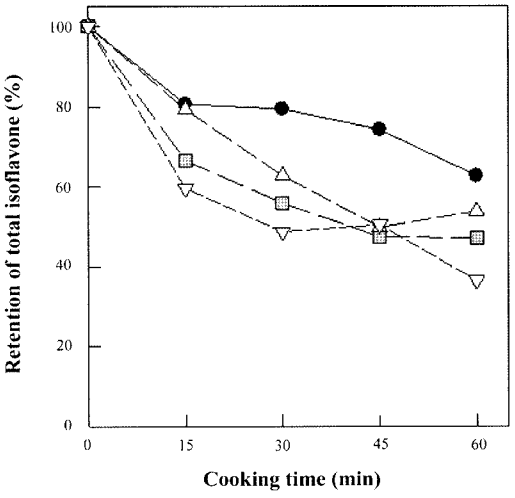

Fig. 4. Changes in the isoflavone content of raw and presoaked soybeans during boiling. ●, Isoflavone content of raw *Hwangkeumkong* after boiling; ■, isoflavone content of presoaked *Hwangkeumkong* after boiling; ▲, isoflavone content of raw *Black* No. 1 after boiling; ▼, isoflavone content of presoaked *Black* No. 1 after boiling at 121°C. Beans were presoaked for 12 hr at 4°C.

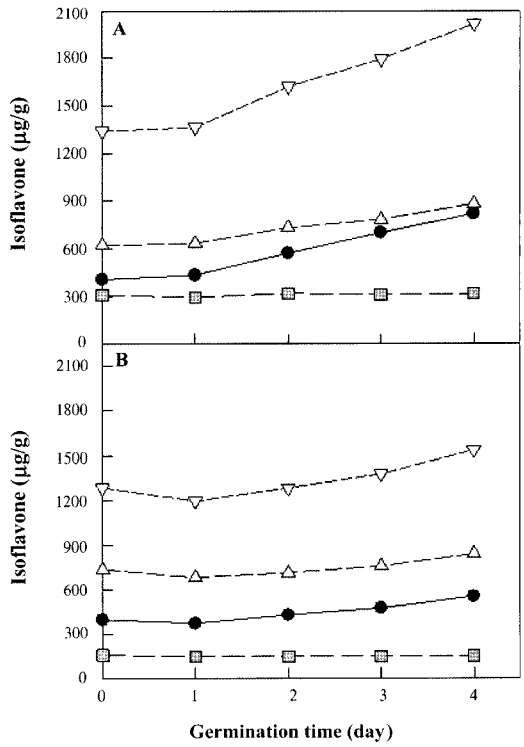

Fig. 5. Effects of germination on the isoflavone composition and content of *Eunhakong* (A) and *Guinunikong* (B). Bean sprouts were cultivated for 4 days at 25°C. ▼, Total isoflavone content; ●, daidzein; ■, glycitein; ▲, genistein.

Preparation of vinegar beans Vinegar beans (soybeans pickled in vinegar) are regarded as healthy product because they contain inactivated anti-nutritional factors such as protease inhibitors and antioxidant activity is increased by acid pickling (26). The *Black* No. 1 and *Hwangkeumkong* samples were exposed to 4 and 7% acetic acid solutions and allowed to ripen for 8 days at 25°C. Subsequently, the isoflavone contents of the vinegar beans were measured, as well as the absorption of acetic acid. After 2 days of exposure to acetic acid, the pH of the vinegar beans was dramatically decreased from 6.6-6.7 to 3.6-4.0; however, as time went on no additional changes in pH were observed. During the first 2 days of exposure to 4% acetic acid, the weights of the samples decreased to 82 and 85% of their initial values for *Hwangkeumkong* and *Black* No. 1, respectively. Between days 2 and 8, the weights of the *Black* No. 1 and *Hwangkeumkong* samples further deceased to 77 and 76% of their initial values, respectively. A similar trend was observed for the samples exposed to 7% acetic acid. The isoflavone content of the vinegar beans produced from *Hwangkeumkong* initially

increased from 1,877 to 1,956 μg/g, and to 2,008 μg/g after 8 days of immersion in 4 and 7% acetic acid, respectively. As shown in Fig. 6, the changes in isoflavone contents of the samples, and the decreases in the sample weights, were each initially around 25%, with no subsequent fluctuations. The isoflavone content of the vinegar beans (885 μg/g) produced from the *Black* No. 1 initially increased to 1,956 and 2,008 μg/g after 8 days of immersion in 4 and 7% acetic acid, respectively. An analysis of the isoflavone content of vinegar beans that were stored for more than 2 months revealed that roughly 70% of the initial components of the beans remained (data not shown), indicating that isoflavone retention is highly viable during the preparation of vinegar beans.

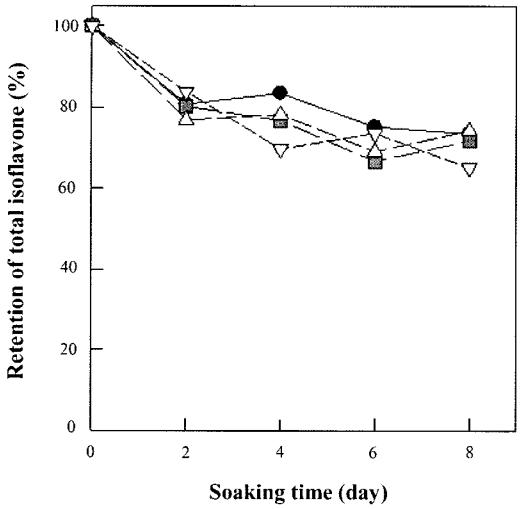

Fig. 6. Changes in the isoflavone content of soybeans soaked in 4 and 7% acetic acid. ●, *Hwangkeumkong* soaked in 4% acetic acid; ■, *Hwangkeumkong* soaked in 7% acetic acid; ▲, *Black* No. 1 soaked in 4% acetic acid; ▼, *Black* No. 1 soaked in 7% acetic acid.

Fermentation Changes in the *meju* isoflavone content according to various parts of the fermented block are shown in Table 1. *Meju* produced by the traditional method using *Hwangkeumkong* contained more than 85% of the isoflavones present in the original beans. The isoflavones in *meju* exist as a converted form of aglycone. In particular, those areas of the *meju* surface showing evidence of intense mold propagation have a higher proportion of aglycone to glycoside forms. Compared to other beans, *Guinunikong*, also known as *yakkong*, has a relatively higher proportion of glycosidic isoflavones. In this study, the *meju* produced with *Guinunikong* beans showed the same tendency toward increased aglycone levels on its surface sites that had excessive mold propagation. Most isoflavones in soybeans are present in the glycoside form and are converted to aglycones during fermentation due to the β-glucosidase activity of microorganisms (27). An increased rate of aglycone production means that a bacterial enzyme participated in glycoside hydrolysis. Figure 7 shows the observed changes in the isoflavone levels for *doenjang* produced with *Guinunikong* and stored at 20°C. The initial amount of isoflavones present was 793 μg/g, and the overall isoflavone content of the sample changed only slightly during a 2 month period; however, the isoflavone content increased between 2 and 4 months, ending at 1,078 μg/g.

Preparation of soybean curd Table 2 shows the yields of the soybean curd, soybean curd residue, and soybean curd whey using three methods of soybean curd production: cold extraction, hot extraction, and whole soybean. The cold extraction method had lower yields than the traditional hot extraction method and produced greater amounts of soybean curd residue. The whole soybean method did not produce soybean curd residue; instead, it produced twice the amount of soybean curd compared to the other two methods, while the amount of soybean curd whey was reduced. Mass balance testing for isoflavones

Table 1. Isoflavone contents of the various parts of fermented *meju*[1)]

Variety	Sampling part	Total isoflavone (μg/g, d.b.)				Aglycone (μg/g, d.b.)				Aglycone/ Glucoside
		Daidzein	Glycitein	Genistein	Total	Daidzein	Glycitein	Genistein	Total	
Hwangkeumkong	Soybean, raw	425.4	146.3	810.1	1381.8	48.7	8.9	151.7	209.6	0.2
	Meju Center of the inner part	413.0	133.7	663.3	1210.0	290.1	88.7	401.6	780.4	0.6
	Meju Outer wall of the surface	451.2	143.3	753.5	1348.0	62.9	15.0	77.9	155.7	0.1
	Meju Center of the surface	413.9	132.6	704.4	1250.9	165.6	46.6	178.2	390.4	0.3
Guinunikong	Soybean, raw	659.9	199.8	917.0	1776.7	0.5	15.3	0.2	16.1	0.1
	Meju Center of the inner part	676.2	143.7	879.0	1698.9	0	0.9	47.5	48.3	0.02
	Meju Outer wall of the surface	746.4	164.5	934.3	1845.2	17.3	8.6	30.2	56.2	0.03
	Meju Center of the surface	668.2	122.4	837.0	1627.6	277.4	31.5	295.4	604.3	0.4

[1)]*Meju* fermented at 29°C for 20 days, and then fermented at 20°C for 30 days after molding.

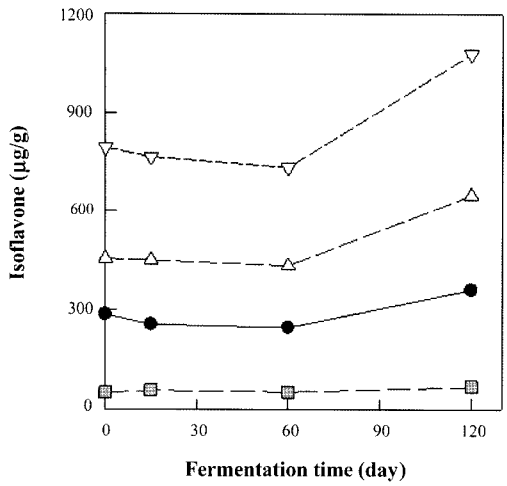

Fig. 7. Changes in the isoflavone content and composition of *doenjang* during fermentation. ▼, Total isoflavone content; ●, daidzein; ■, glycitein; ▲, genistein.

revealed that the production of soymilk caused little isoflavone loss, but that during the subsequent coagulation and molding steps, a considerable amount of isoflavones were drained out as soybean curd whey (Table 3 and 4).

In the hot extraction method, the soybean slurry is filtered after heating to reduce the occurrence of off flavors and to induce enzyme inactivation. In contrast, during the cold extraction method, which is easier to perform, unheated soybean slurry is filtered, heated, and coagulated.

We found that more isoflavones were lost during the cold extraction method than during the hot extraction method. After coagulation of the soymilk with calcium sulfate, we compared the isoflavone contents of the soybean curd residue, soybean curd whey, and soybean curd. For the cold extraction method, only 37.4% of the isoflavones present in the original soybeans remained in the soybean curd, while 30.6% were identified in the soybean curd whey and 19.6% were present in the soybean curd residue. For the hot extraction method, 50.7 % of the isoflavones present in the original soybeans remained in the soybean curd, while 31.6% were found in the soybean curd whey and 14.6% were present in the soybean curd residue. Daidzein and glycitein were among the isoflavones lost in the soybean curd whey, while genistein was largely lost in the soybean curd residue. To increase the retention of isoflavones in soybean curd, reducing the amount of soybean curd whey that is produced should be considered. The production of uncurded, soft, and whole soybean curd would be beneficial for minimizing the loss of isoflavones. Table 5 shows the change in isoflavone content for dried soybeans that had been ground into a fine powder and passed through a 325 mesh. The sample, which lacked soybean curd refuse, contained 80.7% of the isoflavones present in the original soybeans. Only 11.2% of the isoflavones were lost, due to a decrease in the amount of soybean curd whey. Thus, we conclude that the whole soybean method of soybean curd production is best for preserving the nutrients present in soybeans and maximizing the availability of isoflavones.

Most of the processes used to produce soy foods have been optimized for protein content and functionality, as well as for sensory properties. Therefore, it is necessary to develop a production method that can increase the

Table 2. Yields of soybean curd, soybean curd residue, and soybean curd whey according to various soybean curd preparation methods (200 g soybeans)

		Hot extraction method	Cold extraction method	Whole soybean method
Soybean curd	Yield (g)	480.0	378.2	820.0
	Moisture (%)	81.0	77.2	81.7
Soybean curd residue	Yield (g)	243.8	311.4	0
	Moisture (%)	79.8	81.4	-
Soybean curd whey	Yield (mL)	850.0	900.0	240.0
	Solid (°Bx)	3.0	2.8	4.8

Table 3. Changes in the isoflavone content and composition during each step of the hot extraction method

Step	Yield	Isoflavone (mg/soybean 200 g, wet basis)				Retention (%)
		Daidzein	Genistein	Glycitein	Total	
Soybean	200 g	101.4	152.0	21.4	274.8	100.0
Soaking water	1700 mL	0.9	0.4	0.1	1.4	0.5
Boiled soybean slurry	1900 mL	104.4	144.3	19.1	268.0	97.5
Soy milk after filtration	1700 mL	105.4	136.8	19.3	261.5	95.2
Soybean curd residue	243.8 g	14.7	22.8	2.5	40.0	14.6
Soybean curd whey	850 mL	49.9	27.5	9.4	86.8	31.6
Soybean curd	480.0 g	46.5	84.7	8.1	139.3	50.7

Table 4. Changes in isoflavone content and composition during each step of the cold extraction method

Step	Yield	Isoflavone (mg/soybean 200 g, w.b.)				Retention (%)
		Daidzein	Genistein	Glycitein	Total	
Soybean	200 g	101.4	152.0	21.4	274.8	100.0
Soaking water	1700 mL	0.9	0.4	0.1	1.4	0.5
Boiled soy milk after filtration	1700 mL	94.9	127.6	16.8	239.4	87.1
Soybean curd residue	311.4 g	19.8	29.1	4.8	53.9	19.6
Soybean curd whey	900 mL	49.5	26.0	8.6	84.1	30.6
Soybean curd	378.2 g	34.4	63.2	5.3	102.9	37.4

Table 5. Changes in isoflavone content and composition during each step of the whole soybean method

Step	Yield	Isoflavone (mg/soybean 200 g, w.b.)				Retention %
		Daidzein	Genistein	Glycitein	Total	
Soybean powder (passed through 325 mesh)	200 g	116.3	146.5	31.1	293.9	100.0
Boiled soybean slurry	1340 mL	117.8	145.1	30.0	293.0	99.7
Soybean curd residue	0	0	0	0	0	0
Soybean curd whey	240 mL	15.9	13.0	4.0	33.0	11.23
Soybean curd	820 g	88.0	127.9	21.5	237.3	80.74

contents of effective components in the end products. Several studies have investigated changes in isoflavone concentration during the processing of soy-based products (15, 16, 23, 28), but very few data are available for soy foods that are typically used in Korea, especially *meju*, vinegar beans, and soybean curd. The results of this study indicate that vinegar beans and whole soybean curd contain high levels of isoflavones. Further research will be needed to investigate the manufacturing conditions necessary to reduce the loss of isoflavones from soy.

Acknowledgments

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