KOREAN J. FOOD SCI. TECHNOL. Vol. 49, No. 1, pp. 56~62 (2017)
https://doi.org/10.9721/KJFST.2017.49.1.56

KOREAN JOURNAL OF
한국식품과학회지
FOOD SCIENCE AND TECHNOLOGY

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Changes in physicochemical characteristics and nutritional values of soybean, *meju*, and *doenjang* by varying sowing periods

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Abstract The purpose of this study was to determine how seeding time changes the properties of soybean products *meju* and *doenjang*. Soybeans were seeded on the last day of May (5L), on the mid day of June (6M), and on the last day of June (6L), respectively. The 5L soybeans experienced a distinguishing hot and humid climate at the ripening stage, and these climate conditions resulted in smaller seed sizes. Fermentation briskly progressed in all *doenjang* until 120 days, and the 5L *doenjang* exhibited the lowest fermentation efficiency. The 5L soybeans showed the highest GABA, polyphenol contents, and ACE inhibitory activity, and the 6M soybeans showed the highest radical scavenging activity among the groups tested. In *doenjang*, the functional properties generally increased as the aging time approached the 120th day, and the 6M *doenjang* showed the highest functional properties at the 120th day. Therefore, 6M soybeans had the most appropriate characteristics when producing *doenjang*.

Keywords: soybean, seeding time, *doenjang*, aging period, functional nutrients

Introduction

Soybeans are a species of legume native to East Asia. Especially in Korea, Japan, and China, a culture of making various kinds of soybean products specific to the nation exists (1). It is well known that soybeans contain not only vitamins and minerals but also useful bioactive substances, including isoflavones, saponins, phytosterols and oligosaccharides (2). *Meju* is a starter form for manufacturing soybean paste (*doenjang*), soy sauce (*ganjang*), and red pepper paste (*gochujang*). Various types of *meju* exist according to region, purpose, ingredient, shape, and other factors. *Meju* can be divided into two major categories: the improved and traditional types. For making traditional *meju*, steamed soybeans are molded to specific shapes, fixed with a rice straw, and fermented with microorganisms which exist naturally in the rice straw and air. However, when making improved *meju*, refined microorganisms like the *Aspergillus* species are inoculated (3). *Doenjang* is made from *meju*, and it takes an aging process for the production of its unique flavor, which is produced by decomposed carbohydrates, proteins, and fats. Many researches confirmed that the physiological activity of fermented soybean products came from the secondary metabolites of microbes; also, some of components such as phenolic acid, isoflavones, and oligo-peptides showed antioxidant activity (4). *Doenjang* is known to possess functional properties like nitrate elimination, the reduction of blood pressure and of the risk of hyperlipidemia and diabetes, as well as having anti-mutagenic, anti-carcinogenic, and immunity-improving properties (5).

Yields of vegetables are strongly associated with the conditions of the environment in which they are grown. There have been a lot of studies focused on identifying the optimum conditions of soybean culture. According to recent studies, not only the yields but also the nutritional composition and phytochemical components of soybeans can vary based on environment factors even if they are the same species because of changes in assimilation and enzyme activity (6). Furthermore, these changes in the compositions of soybeans can determine the qualities of the products manufactured from them. Studies of soybean products produced from different seeding conditions were conducted for tofu (7), bean sprouts (8), *doenjang* (9), *ganjang* (6), and other foods. However, studies on soybean characteristics with different sowing times, and their influence on fermentation properties or the nutritional value of *doenjang* are still insufficient for domestic use.

The goal of this research was to find out how seeding time changes the properties of soybeans, and how soybeans with different seeding times can influence the properties of *meju* and *doenjang*. The sowing periods of soybeans were divided into three conditions under proper sowing periods recommended by RDA. *Meju* and *doenjang* were made from soybeans by the traditional Korean manufacturing method. The physical characteristics of soybeans were examined; for soybean, *meju*, and *doenjang*, proximate compositions, fermentation indicators (pH, titratable

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Received July 20, 2016; revised September 26, 2016; accepted September 26, 2016

acidity, total of free amino acids, reducing sugar, and brown color), functional nutrients contents (isoflavone, GABA, and polyphenol), antioxidant activity, and ACE inhibitory activities were analyzed.

Materials and Methods

All chemicals used for experiments were obtained from Sigma-Aldrich (St. Louis, MO, USA). Soybeans were provided by the Rural Development Administration (RDA, Milyang, Korea) of Korea.

Plant materials and growth conditions

Soybeans (*Glycine max* L.), cv. Daewon were grown in an outdoor farm in *Jinkyomyeon,* Hadong, Korea (35°06'N, 127°75'E, 20 m above mean sea level). A prevalent sowing time of soybeans which recommended by the RDA is from the last of May to the mid of June. Under this period, sowing times were subdivided to three. Soybeans were planted on May 30th (the last day of May, 5L), June 15th (the middle of June, 6M), and June 30th (the last day of June, 6L) in 2010, each of the cultures was grown for 120 d. The methods of cultivation followed general cultivation conditions which were recommended by the RDA (10). The meteorological conditions are calculated with data of the Meteorological Administration of Korea.

Preparation of *meju* and *doenjang*

Meju and *doenjang* were made using traditional processing methods of Korea consulting literatures with some modifications (11). 7 kg of soybeans were washed with water, and then they were boiled in a caldron filled with 15 L of water for 6 h. The boiled soybeans were placed in a colander for 30 min for drainage, and then they were mashed up with a large mortar. The mashed soybeans were shaped with a mold (8×12×20 cm), and they were dried for 1 week at 30°C. After collecting *meju* samples, *meju* was bundled up with rice straw, and fermented for 60 days at 25°C before used for making *doenjang*. Fermented *meju* were washed with fresh water. 7 kg of washed *meju*, and 15 L of 17% brine solution were put in pottery jars and stored for 60 days outdoors. After 60 days, solids were separated and were put in pottery jars for ripening. Pottery jars were placed outdoors in Yangpyeong, Korea (37°35'N, 127°34'E), and *doenjang* was collected on the 0, 60th, and 120th days.

Physical characteristics of soybeans

Defective soybeans were removed before commencing with the experiments. The weight per 100 seeds was measured with ten replicates. For determining the shape of the soybeans, the length, the width, the thickness, and the length of hilum were measured with a caliper with 30 replicates.

Proximate compositions

Crude fat, moisture, ash, and crude protein contents were determined by AOAC methods (12). The carbohydrate content was calculated by a difference of 100 and the sum of fat, protein, moisture, and ash.

Analysis of fermentation indicators (pH, titratable acidity, brown color, reducing sugar, and total of free amino acids contents) and GABA contents

The pH value and titratable acidity were measured by using the methods of Moon *et al.* (13) with pH meter (Seven Easy pH meter, Mettler-Toledo, OH, USA). The brown color index was measured with methods by Shaul *et al.* (14) The amount of reducing sugar was measured with the dinitrosalicylic acid (DNS) method. The total of free amino acids and γ-aminobutyric acid (GABA) contents were measured with HPLC (Agilent 1200LC, Agilent Technol., CA, USA) by methods of Godel *et al.* (15).

Analysis of isoflavone contents

The contents of isoflavone were determined by reversed phase HPLC analysis (Ultimate 3000, Thermo Scientific Dionex, Hudson, NH, USA). Analysis was conducted based on the method of Lee *et al*. (16).

Analysis of polyphenol content, DPPH free radical scavenging activity, and ACE inhibitory activity of 80% methanol extracts

At the beginning, 80% methanol extracts were made. 10 g of freeze-dried powder were put into a flask and 100 mL of 80% methanol were added (1:10, w:v). The extraction was conducted with a shaker (SI600R, Lab Companion, Dajeon, Korea) at 200 rpm for 12 h. The solvent was filtered with No. 1 filter paper (Whatman, Buckinghamshire, UK), and the resulting powders were extracted again with 80% methanol two times. The collected solvents were distilled off in a rotary evaporator (RE111, Büchi, Flawil, Switzerland) at 55°C, and then the remaining solution was freeze-dried. The total polyphenol contents were determined with the Folin-Ciocalteu method (17). The DPPH free radical scavenging activity was measured using the method of Brand-Williams *et al.* (18). A spectrophotometer (Optizen 2120UV, Mecasys, Dajeon, Korea) was used when the absorbance of the samples was measured. Angiotensin-converting-enzyme (ACE) inhibitory activity was determined by using the method of Kwon and Shin (19).

Statistical analysis

Data were expressed as the mean±standard deviation (SD) of triplicate measurements. One-way analysis of variance (ANOVA) and Duncan's multiple-range test ($p<0.05$) were used for statistical analysis using IBM SPSS statistics version 21.0 for Windows (SPSS Inc., Chicago, IL, USA).

Results and Discussion

Meteorological conditions and physical characteristics of soybeans with different sowing times

The meteorological conditions are shown in Table 1. The development stages of soybeans are largely categorized into four stages in this study. These four stages are the seedling & early vegetative stage, vegetative growth stage, reproductive (flowering

Table 1. The meteorological conditions of soybeans under different sowing times

Sowing times[1)]	Stage of development	Period	Temperature (ºC)			Sum of cumulative temperature (ºC)	Sum of cumulative radiation (MJ/m^2)	Sum of amount of rainfall (mm)
			Mean	Min	Max			
5L	Seedling & Early vegetative	May.31-Jun.29	22.6	17.4	29.3	679.2	555.3	43.2
	Vegetative	Jun.30-Jul.29	25.3	22.3	29.9	759.8	440.9	424.2
	Reproductive	Jul.30-Aug.28	27.1	23.8	32.1	813.7	441.8	459.0
	Ripening	Aug.29-Sep.27	23.0	19.3	29.2	690.5	425.5	224.9
	Whole life	May.31-Sep.27	24.5	20.7	30.1	2943.2	1863.5	1151.3
6M	Seedling & Early vegetative	Jun.16-Jul.15	24.3	20.7	29.2	728.4	459.9	271.2
	Vegetative	Jul.16-Aug.14	26.7	23.4	31.5	801.0	457.6	363.2
	Reproductive	Aug.15-Sep.13	26.0	22.9	31.0	779.7	398.2	507.0
	Ripening	Sep.14-Oct.13	18.7	14.0	25.9	560.5	433.3	52.4
	Whole life	Jun.16-Oct.13	23.9	20.3	29.4	2869.6	1749.0	1193.8
6L	Seedling & Early vegetative	Jul.1-Jul.30	25.3	22.3	29.9	759.8	440.9	424.2
	Vegetative	Jul.31-Aug.29	27.1	23.8	32.1	813.7	441.8	459.0
	Reproductive	Aug.30-Sep.28	23.0	19.3	29.2	690.5	425.5	224.9
	Ripening	Sep.29-Oct.28	14.6	9.5	21.9	438.8	398.1	66.5
	Whole life	Jul.1-Oct.28	22.5	18.7	28.3	2702.8	1706.3	1174.6

[1)]5L, Soybeans seeded on May 30th; 6M, Soybeans seeded on June 15th; 6L, Soybeans seeded on June 30th

Table 2. The shape and the weight per 100 seeds of soybeans under different sowing times

Sowing times[1)]	Seed size (mm)				G (L/W)	Weight per 100 seeds (g)
	Length (L)	Width (W)	Thickness (T)	Hilum (H)		
5L	7.34±0.64$^{b2)}$	7.17±0.53^{b}	6.25±0.59^{b}	2.85±0.26^{b}	1.18±0.07	23.9±0.7^{b}
6M	7.86±0.53^{a}	7.58±0.44^{a}	6.63±0.43^{a}	3.01±0.25^{a}	1.19±0.08	25.7±0.5^{a}
6L	7.70±0.51^{a}	7.45±0.45^{a}	6.56±0.37^{a}	3.03±0.19^{a}	1.17±0.06	25.6±0.6^{a}

Results are shown as mean±SD, measurements were replicated for ten times
[1)]5L, Soybeans seeded on May 30th; 6M, Soybeans seeded on June 15th; 6L, Soybeans seeded on June 30th
[2)]Different superscripts within columns are significantly different at $p<0.05$

and early pod) stage, and ripening stage. Each stage takes 30 days, and different development stages have differences in climate conditions. The RDA (10) reported that the minimum level of the sum of the cumulative temperatures of soybeans is 2500-3000ºC, and all of the soybeans fulfilled this requirement. In the case of the mean, minimum and maximum temperatures, the sum of the cumulative temperatures, and rainfall amounts, there were not distinctive differences for the entire lives of the soybeans. However, in terms of subdivided development stages, 5L showed distinguished hotter and more humid climate conditions at the ripening stage. For the sum of the cumulative radiation, 5L showed the highest values during its entire life.

The physical characterization of the seeds and the weight of one hundred seeds are shown in Table 2. The L/W values were almost equal, which indicates that the overall shapes of the seeds were identical. However, 5L exhibited much smaller seed size values than those of 6M and 6L ($p<0.05$). Similarly, the weight of one hundred seeds of 5L (23.9±0.7 g) was the lightest ($p<0.05$). The larger gap between the maximum and minimum temperatures during the day and night at the ripening stage could help gain higher yields, and at the same stage, high temperature and a large amount of rainfall could decrease the quality of the seeds (10). 5L showed a smaller gap between the maximum and minimum temperatures, and it also showed hot and humid climate conditions at the ripening stage, so it is expected that these meteorological conditions resulted in smaller and lighter seeds.

Proximate compositions of soybeans, *meju*, and *doenjang*

The proximate compositions of soybean, *meju*, and *doenjang* are shown in Table 3. The moisture, crude protein, and crude ash of soybeans did not show differences in 5L, 6M, and 6L ($p>0.05$), but 5L showed significantly lowest amounts for lipids ($p<0.05$). Wolf *et al.* (20) said that the oil quantity of soybeans is closely connected with temperature. Like the preceding, it is expected that the smaller oil contents are related with the climate conditions of 5L, and the lighter seed weight is caused from the loss of oil accumulation. For *doenjang*, the crude protein content was highest for 5L, and the crude lipid content was highest for 6L ($p<0.05$). Moisture compositions did not shown significant differences in 0 day *doenjang*, and they were gradually lowered as storage time increased ($p<0.05$). The crude protein and lipid composition progressively increased during aging for all *doenjang*, which resulted from a loss of moisture

Table 3. Proximate compositions of soybean, *meju*, and *doenjang* with different sowing times (unit: %, wet basis)

	Sowing times[1)]	Soybean	*Meju*	*Doenjang* (Days)		
				0	60	120
Moisture	5L	6.23±0.10	9.63±0.10[b2)]	60.66±0.66[A]	55.48±0.27[B]	54.86±0.08[C]
	6M	6.27±0.12	10.53±0.49[a]	60.79±0.51[A]	55.01±0.72[B]	55.41±0.46[C]
	6L	6.15±0.08	9.47±0.14[b]	60.68±0.28[A]	55.86±0.47[B]	55.52±0.26[C]
Crude protein	5L	38.38±0.49	39.14±0.34[b]	12.57±0.17[aC]	12.89±0.12[bB]	13.61±0.15[aA]
	6M	38.89±0.06	42.94±0.64[a]	12.19±0.09[bB]	13.16±0.13[aA]	12.90±0.22[bA]
	6L	39.37±0.47	43.03±0.06[a]	12.28±0.13[bB]	12.89±0.10[aA]	13.01±0.17[bA]
Crude lipid	5L	12.43±0.75[b]	14.20±0.22[b]	7.03±0.19[bB]	7.55±0.04[A]	7.63±0.36[bA]
	6M	14.36±1.12[a]	17.84±0.22[a]	7.15±0.25[b]	7.73±0.72	7.77±0.90[b]
	6L	15.92±0.31[a]	17.33±0.64[a]	7.98±0.15[a]	8.22±0.71	8.38±0.48[a]
Crude ash	5L	5.38±0.07	5.47±0.18	13.92±0.66[B]	16.37±0.20[aA]	15.73±0.65[A]
	6M	5.29±0.18	5.41±0.06	13.61±0.68[B]	15.98±0.31[abA]	15.29±0.80[A]
	6L	5.42±0.12	5.26±0.15	13.30±0.11[B]	15.70±0.11[bA]	15.46±0.65[A]
Carbohydrates	5L	37.58	31.56	5.82	7.71	8.17
	6M	35.19	23.28	6.71	8.12	8.63
	6L	33.14	24.91	5.76	7.33	7.63

Results are shown as mean±SD
[1)]5L, Soybeans seeded on May 30th; 6M, Soybeans seeded on June 15th; 6L, Soybeans seeded on June 30th
[2)]Different superscripts within columns (a-c) or rows (A-C) are significant differences at $p<0.05$

(21). The crude ash contents did not show significant differences with various sowing times. Compared to soybeans and *meju*, the crude ash content of *doenjang* highly increased because of added salt during the process of making *doenjang*.

pH, titratable acidity, reducing sugar content, total of free amino acids content, and brown color value

The results of the analyses of pH, titratable acidity, reducing sugar content, total of free amino acids content, and brown color value are shown in Table 4, and these values serve as fermentation indicators of *doenjang*.

There were no distinctive differences in the pH values of soybeans and titratable acidity ($p<0.05$). For *meju* and *doenjang*, 6M showed the highest pH value, 5L showed the lowest pH value ($p<0.05$), and the titratable acidity was almost reversely affected by pH values. The decreasing pH value and increasing titratable acidity are due to the creation of organic acids by microorganisms in *doenjang* like lactobacillus, and these organic acids can positively affect the taste and aroma of fermented products (22).

The reducing sugar contents of the soybeans were 2.01-2.28%, and 6L showed the lowest content ($p<0.05$). For *meju*, the reducing sugar contents were 4.38±0.11% (6L), 4.10±0.08% (6M), and 3.20±0.07% (5L) in that order. The reducing sugar contents of 0 day *doenjang* were lower than those of *meju*, and 6M *doenjang* showed the highest content, while 6L showed the lowest content ($p<0.05$). The reducing sugar contents of *doenjang* all decreased as the aging time increased. 6L *doenjang* showed a drastic reduction of reducing sugar contents of *meju* to 0 day *doenjang*, and it is expected that this drop occurred when *meju* was fermented for 60 days by active metabolic reactions.

6M showed the lowest free amino acids contents (191.44±2.14 mg/100 g) for soybeans ($p<0.05$). However, 6M exhibited the highest free amino acids contents for *meju*, and 6L exhibited the highest value for *doenjang* ($p<0.05$). The total of free amino acids contents in 5L, 6M, and 6L *doenjang* increased as the aging times increased, and these results indicate the progression of fermentation. Free amino acids are strongly involved in producing taste per se, and they could be materials of other metabolites associated with the flavors of foods (23).

The brown color values of soybeans were 0.32-0.40, and the value of 6L was the highest, 5L, followed by that of 6M. The brown color values of *doenjang* were 0.39-0.50 at the 0 day, 0.51-0.65 at the 60th day, and 0.51-0.77 at the 120th day. For *doenjang* at the 120th day, 6M showed the highest brown color value ($p<0.05$), and its value was 0.77±0.02. As aging time increased, the brown color value of *doenjang* became higher. These changes seem to be caused by Maillard reaction and enzymatic browning reaction by the byproducts of microorganisms during ripening. The microorganisms in *doenjang* contain protease and amylase, and these enzymes make profuse breakdown products like oligopeptides and reducing sugars, which produce Maillard reaction products (MRPs) (24). The study of Lee *et al*. (25) also showed that the brown color value increased as the storage period of *doenjang* increased. The brown color value of 6M *doenjang* was strikingly higher than the brown color values of others, which may have resulted from the highest reducing sugar contents of 6M.

The isoflavone contents of the free amino acids of soybeans, *meju*, and *doenjang*

Isoflavones can ease the side effects of menopause, reduce the risk of cardiovascular disease, and protect against some

Table 4. pH, titratable acidity, reducing sugar, total free amino acids, and brown color of soybean, *meju*, and *doenjang* with different sowing times

	Sowing times[1)]	Soybean	*Meju*	*Doenjang* (Days)		
				0	60	120
pH	5L	6.70±0.01	6.37±0.01[c2)]	5.13±0.02[cA]	4.83±0.02[cB]	4.73±0.01[cC]
	6M	6.65±0.05	6.48±0.02[b]	5.84±0.01[aA]	5.24±0.03[aB]	5.19±0.01[aC]
	6L	6.44±0.38	6.64±0.02[a]	5.34±0.01[bB]	5.95±0.01[bA]	4.91±0.02[bC]
Titratable acidity	5L	0.96±0.03	1.23±0.04[a]	0.97±0.01[aB]	1.44±0.01[aA]	1.43±0.02[aA]
	6M	1.02±0.01	1.09±0.02[b]	0.80±0.01[cC]	1.24±0.05[cB]	1.33±0.03[bA]
	6L	1.01±0.04	1.31±0.07[a]	0.87±0.02[bB]	1.31±0.02[bA]	1.31±0.01[bA]
Reducing sugar (%)	5L	2.27±0.08[a]	3.20±0.07[c]	2.86±0.21[bA]	2.35±0.08[bB]	2.17±0.09[bB]
	6M	2.28±0.04[a]	4.10±0.08[b]	3.92±0.12[aA]	3.28±0.02[aB]	3.03±0.01[aC]
	6L	2.01±0.12[b]	4.38±0.11[a]	1.81±0.14[cA]	1.06±0.02[cB]	0.97±0.07[cB]
Total free A.A (mg/100 g)	5L	212.86±1.15[a]	333.96±9.14[b]	1344.20±36.36[bC]	1970.82±1.03[bB]	2349.15±33.88[bA]
	6M	191.44±2.14[b]	436.54±12.08[a]	1478.09±41.88[aC]	2265.42±42.05[aB]	2643.03±21.53[aA]
	6L	224.46±2.45[a]	359.42±12.17[b]	1542.93±13.05[aC]	2212.31±0.55[aB]	2618.27±32.97[aA]
Brown color	5L	0.36±0.03[b]	0.40±0.00[a]	0.39±0.02[cB]	0.51±0.00[bA]	0.51±0.03[bA]
	6M	0.32±0.01[c]	0.37±0.00[b]	0.50±0.02[aC]	0.65±0.02[aB]	0.77±0.02[aA]
	6L	0.40±0.00[a]	0.40±0.02[a]	0.44±0.03[bC]	0.51±0.00[bB]	0.56±0.02[bA]

Results are shown as mean±SD
[1)]5L, Soybeans seeded on May 30th; 6M, Soybeans seeded on June 15th; 6L, Soybeans seeded on June 30th
[2)]Different superscripts within columns (a-c) or rows (A-C) are significant differences at $p<0.05$

Table 5. Isoflavone contents of soybean, *meju*, and *doenjang* with different sowing times (unit: mg/100 g, dry basis)

	Sowing times[1)]	Soybean	*Meju*	*Doenjang* (Days)		
				0	60	120
Daidzein	5L	70.61±4.14	54.06±4.28[a2)]	60.60±1.45[aA]	45.91±3.22[bB]	56.81±3.78[aA]
	6M	75.93±2.38	40.98±1.14[b]	53.24±1.94[bA]	41.58±2.13[bB]	51.04±1.34[bA]
	6L	71.82±2.57	45.63±3.59[b]	58.63±0.37[aAB]	56.55±1.60[aB]	62.13±2.81[aA]
Genistein	5L	54.11±4.65	45.67±1.82[a]	51.29±0.06[bA]	44.03±3.81[bB]	49.59±2.62[aA]
	6M	58.97±1.86	36.59±0.63[c]	48.68±0.14[cA]	36.68±3.38[cC]	41.95±3.95[bB]
	6L	58.16±0.80	42.16±1.73[b]	54.15±0.17[aA]	50.68±1.18[aB]	54.10±0.61[aA]
Total isoflavone (Daidzein+ Genistein)	5L	124.72±8.73	99.73±6.10[a]	111.86±1.39[aA]	89.93±6.99[bB]	106.40±5.40[bA]
	6M	134.90±3.97	77.57±0.73[c]	101.92±3.57[bA]	78.26±4.69[cB]	92.99±5.24[cA]
	6L	129.99±3.29	87.80±5.32[b]	112.77±0.52[aA]	107.22±2.78[aB]	116.24±3.19[aA]

Results are shown as mean±SD
[1)]5L, Soybeans seeded on May 30th; 6M, Soybeans seeded on June 15th; 6L, Soybeans seeded on June 30th
[2)]Different superscripts within columns (a-c) or rows (A-C) are significant differences at $p<0.05$

hormone-dependent cancers (26). Daidzein and genistein are major isoflavones of soybeans, and the total isoflavone contents of soybeans in Korea were distributed to 46-418 mg/100 g, and the 5L, 6M, and 6L soybeans were all within this boundary (27). Tsukamoto *et al.* (28) reported that the isoflavone contents of soybeans decreased as the temperature increased and when there were excessive rainfall amounts. In this study, there were no significant differences in the isoflavone contents of soybeans. In the case of *meju* and *doenjang*, the isoflavone contents all decreased compared with soybeans ($p<0.05$), and this phenomenon is attributed to the loss of isoflavones during processing *meju* and *doenjang* (29). For *doenjang*, at all storage period conditions, 6M exhibited the lowest total of isoflavone amounts ($p<0.05$). The isoflavone contents of *doenjang* decreased during 0 to 60 days, and they increased during 60 to 180 days ($p<0.05$). The decrease of isoflavone is related to the decomposition of isoflavone aglycones (daidzein and genistein), and the restoration of isoflavone contents is involved to transform the isoflavone glycosides to isoflavone aglycones by microorganisms (29).

Functional compounds contents (GABA and polyphenol), DPPH radical scavenging activities, and ACE inhibitory activities of soybeans, *meju*, and *doenjang*

GABA is known for its functionalities such as promoting brain function and the metabolism of alcohol, or reducing neutral fat and sugar in the blood (30). The contents of GABA are shown in Table 6. For soybeans, 5L presented the highest contents and 6M showed the lowest contents ($p<0.05$). This tendency also appeared for *meju* and 0 day *doenjang*, but as

Table 6. The contents of GABA, polyphenol content, DPPH radical scavenging activity, and ACE inhibitory of 80% MeOH extract of soybean, *meju*, and *doenjang*

	Sowing times[1)]	Soybean	*Meju*	*Doenjang* (Days)		
				0	60	120
GABA (mg/100 g)	5L	16.93±0.10[a2)]	9.30±0.21[a]	7.54±0.21[a]	8.18±0.52[b]	7.53±0.28[a]
	6M	11.32±0.14[c]	7.74±0.40[b]	6.05±0.84[b]	7.07±0.25[c]	7.03±0.21[b]
	6L	15.75±0.16[b]	5.40±0.61[c]	6.79±0.06[bC]	9.18±0.59[aA]	7.86±0.12[aB]
Polyphenol (mg GAE eq./100 g)	5L	1080.84±41.20	1368.26±32.88	736.23±30.97[C]	961.86±4.38[A]	940.90±10.75[B]
	6M	1013.65±35.24	1435.04±98.93	802.42±49.75[B]	974.00±64.60[A]	977.42±50.20[A]
	6L	1007.07±48.21	1408.58±76.31	745.10±83.32[B]	891.57±64.11[AB]	946.67±36.14[A]
DPPH (mg VCEAC/g)	5L	1.34±0.03[c]	1.88±0.01	1.02±0.00[cC]	1.06±0.01[cB]	1.19±0.01[bA]
	6M	1.55±0.01[a]	1.88±0.01	1.11±0.01[aC]	1.24±0.01[aB]	1.26±0.00[aA]
	6L	1.42±0.04[b]	1.83±0.04	1.06±0.01[bB]	1.11±0.01[bB]	1.13±0.01[cA]
ACE inhibitory (%)	5L	49.70±0.61[a]	67.51±0.96[b]	54.79±3.81[B]	61.33±2.11[A]	62.39±2.41[A]
	6M	47.93±1.30[a]	66.04±0.65[b]	54.49±2.40[B]	56.95±3.74[B]	63.33±0.42[A]
	6L	43.96±1.41[b]	70.83±1.35[a]	55.50±3.78[B]	55.50±3.78[B]	62.09±4.60[A]

Results are shown as mean±SD
GABA contents, polyphenol contents, and DPPH radical scavenging activity are expressed with dry basis
mg GAE eq., mg gallic acid equivalent; mg QE eq., mg quercetin equivalent
[1)]5L, Soybeans seeded on May 30th; 6M, Soybeans seeded on June 15th; 6L, Soybeans seeded on June 30th
[2)]Different superscripts within columns (a-c) or rows (A-C) are significant differences at $p<0.05$

the aging time increased, 6L *doenjang* presented the highest GABA content ($p<0.05$). Only 6L *doenjang* showed significant changes on GABA contents during fermentation. GABA contents of *doenjang* were increased until 60 days, then, GABA contents were decreased during 60 to 120 days. GABA could be largely produced by lactobacillus, so the GABA content typically increases as the aging time increases (31). It is expected that this contrasting result is caused from differences in the manufacturing and storage conditions of *doenjang*.

As revealed in Table 6, there were no significant differences in the polyphenol contents of soybeans, *meju*, and *doenjang* with different sowing times. The amount of polyphenol in *meju* was greater than that in soybeans, but there was a decrease in the polyphenol contents after the *meju* was processed to *doenjang* by mixing it with a brine solution. The contents of the polyphenol of 5L, 6M, and 6L *doenjang* increased after 60 days of fermentation, and the increase of polyphenol contents continued until the 120th day in 6L ($p<0.05$). Rhee and Cheigh (32) also reported that the polyphenol contents increased during fermentation for *doenjang*.

The results of DPPH radical scavenging activities are shown in Table 6. 6M showed the highest radical scavenging activities for soybean and *doenjang* within a given aging period ($p<0.05$). 5L, 6M, and 6L all represented the strongest radical scavenging activities for *meju* (1.83-1.88 mg VCEAC/g) compared with soybeans (1.34-1.55 mg VCEAC/g) and *doenjang* (1.023-1.26 mg VCEAC/g) ($p<0.05$). DPPH radical scavenging activities increased as the aging increased for all *doenjang* ($p<0.05$). Maillard *et al*. (33) said that the antioxidant activities of foods made of vegetables are mainly caused from phenol compounds. This inclination also appeared in this study, but the radical scavenging activity of the 6M *doenjang* was strikingly higher though there were no significant polyphenol differences in the *doenjang* of 5L, 6M, and 6L. Thus adscititious synergism with other functional compounds seemed to be at work, and it was expected that MRPs were some of such compounds (34).

The ACE inhibitory results are represented in Table 6. The ACE inhibitor can drop blood pressure by decreasing aldosterone secretion and increasing bradykinin, which enlarges blood vessels (35). 6L significantly showed the lowest ACE inhibitory activities in soybeans, but 6L significantly showed the highest value in *meju* ($p<0.05$), and there were no ACE inhibitory differences in 5L, 6M, and 6L for *doenjang*. The ACE inhibitory activity of all *doenjang* increased as the aging period increased from 0 to 120 days ($p<0.05$). The main compounds in *doenjang* which influence ACE inhibitory activity were known to be peptides produced by hydrolyzed protein (36). Free amino acids and amino-type nitrogen contents (data not shown) in *doenjang* sharply increased during ripening. No *et al.* (36) also reported that the ACE inhibitory activity of *doenjang* made with isolated *nuruk* increased during fermentation.

Conclusions

At the conclusion, there were no distinctive differences in the meteorological conditions for the entire lives of 5L, 6M, and 6L. However, 5L showed a distinguishing hot and humid climate at the ripening stage, and these climate conditions impinged on smaller seed sizes of 5L, the result of decreased lipid accumulation. Fermentation briskly progressed in all *doenjang* until 120th days, and the 5L *doenjang* exhibited the lowest fermentation efficiency. Isoflavone contents did not show a significant gap by different sowing times. For soybeans, 5L showed the highest GABA, polyphenol contents and ACE inhibitory activity, and 6M showed the highest radical scavenging activity. In *doenjang*, the functional properties generally increased as the aging time approached the 120th day, and on the 120th day, the 6M *doenjang* was shown to have the highest functional properties. Though 5L soybeans possessed some nutritional values high, their fermentation efficiency in *doenjang* was the lowest. 6M soybeans presented more yields than 5L, and the functional properties of *doenjang* was also fine. For this reason, 6M soybeans had the most appropriate characteristics when made *doenjang*.

Acknowledgment

This work was carried out with support of "Cooperative Research Program for Agriculture Science & Technology Development (Project No. PJ006376)" Rural Development Administration, Republic of Korea.

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