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Quality Characteristics of *Meju* According to Germination Time of Raw Soybean (*Glycine max: Hwanggeumkong*)

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Abstract This study was conducted to observe quality characteristics of whole soybean *meju* fermented with germinated soybean (*Glycine max*: *Hwanggeumkong*). The germination rate after 24 hr was 23.0±1.2%, then increased rapidly to 90.2± 1.3% at 36 hr of germination, and finally reached a level of 99.4±0.3% at 60 hr of germination. It was confirmed that the total isoflavone content immediately after soaking was 100.1 mg%, increasing during the beginning of the germination process; it continued to increase to 114.0 mg% by 24 hr of germination, but decreased thereafter. The isoflavone content at 60 hr of germination was 101.6 mg%. A total of 6 organic acids were detected, and total organic acid content ranged from 963.1-1,145.3 mg%. Differences based on the degree of germination in the raw material were insignificant. The free amino acid levels of the whole soybean *meju* made from non-germinated soybeans and from soybeans that had germinated 48 hr were 2,580.9 and 2,519.7 mg%, respectively. The content of glutamic acid was highest followed by aspartic acid, lysine, leucine, and proline.

Keywords: germination, soybean, whole soybean *meju*, organic acid, amino acid, isoflavone

Introduction

Proteins and carbohydrates that accumulate in seeds during development are not only essential reserves that support germination and early seedling growth in plants, they also serve as major food and nutrient sources for humans and animals (1). Soybeans, in particular, supply a major portion of the world's demand for vegetable oil and protein (2).

In addition to soybean seeds, germinated soybean sprouts are served as staple vegetables and used in salads, soups, and side dishes in many Asian countries (3). In the natural environment, seed sprouts survive during germination by enhancing their defensive responses through the biosynthesis of phenolics (4). The germination process may cause changes in the nutrients found in soybeans, including functional substances, through aerobic respiration and biochemical metabolism. Sprouting also removes antinutrients, such as enzyme inhibitors in the seeds, thus making sprouts safe for human consumption (5).

The majority of research on germinated soybeans has focused on soybean sprouts, which were typically germinated for more than 5 days (7-9). Lee *et al.* (6) investigated the isoflavone content and quality improvement of soymilk produced from germinated soybeans. *Meju* made with soybeans is an important starter cake used in the preparation of traditional soy sauce and soybean paste, and has been a basic material dominating the taste and flavor of these fermented foods (10).

In this study, the quality characteristics of *meju*, including taste compounds and isoflavone contents, were studied according to the germination time of raw soybeans in an effort to provide basic research for the development of functional foods using germinated soybeans.

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Materials and Methods

Materials The soybeans (*Glycine max*: *Hwanggeumkong*) used in this experiment were purchased from Soyventure Co., Ltd. (*Daegu, Korea)*, which were harvested in 2005. The soybean proximate composition was moisture 8.3%, ash 4.8%, crude protein 30.0%, crude fat 19.3%, crude fiber 5.7%, and nitrogen free extract 31.9%.

Germination The washed soybean seeds were soaked in 20°C water for 4 hr and then transferred into a culture container (25×25×30 cm); the culture containers were placed in a thermostat dark house. The soaked soybean seeds were cultivated for 60 hr under a top-irrigation system commercially used for soybean sprout production. Irrigation was given for 3 min every 2 hr with underground water. The average temperature regime inside the culture house during the experiments was 20 to 25°C.

Preparation of whole soybean *meju* The method used for producing the whole soybean *meju* made with germinated soybeans, was based on those of Kim *et al.* (11), and is described in Fig. 1. First, the germinated soybeans were drained for 1 hr, put into an Erlenmeyer-flask, steamed for 40 min at 121°C, and cooled down to 40 °C. Then *Aspergillus oryzae,* isolated and identified from a commercial *meju*, was inoculated to the steamed and germinated soybeans at 10^6 spores/g. The whole soybean *meju* made with the germinated soybeans was prepared by

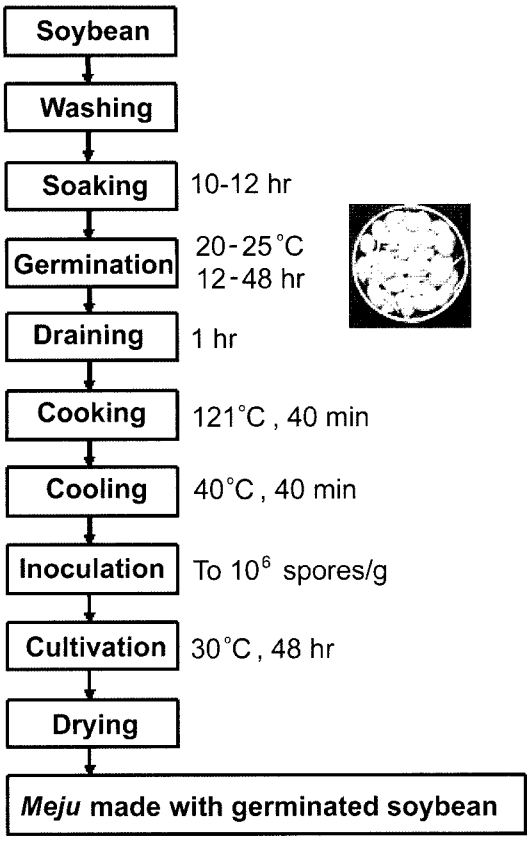

Fig. 1. Protocol for the preparation of *meju* made with germinated soybeans.

culturing the inoculated steamed soybeans at 30°C for 72 hr and drying with a hot air dryer (SGO-100; Sunggun, Korea) for 2 days.

Measurement of germination characteristics Total yield was expressed as the percentage of harvested sprout weight relative to the dry seed weight, as described in Eq. (1). The germination ratio was expressed as the percentage of the number of germinated soybeans relative to the number of total soybeans, as shown in Eq. (2). Twenty germinated *Hwanggeumkong* soybeans were selected randomly according to germination time, and sprout lengths were measured with a vernier caliper (5/100 m, Mitutoyo, Gawasaki, Japan).

Total yield (%)
= [weight of germinated soybeans (g) / weight of dried soybeans (g)] × 100 (1)

Germination ratio (%)
= [The number of germinated soybeans (EA) / Total soybeans (EA)] × 100 (2)

Color measurement The *meju* color measurements were taken with a chromameter (Chromameter CR 300; Minolta, Osaka, Japan) and were calibrated with a white standard plate (L = 97.51, a = -0.18, b = +1.67).

pH measurement pH measurements were carried out with a pH meter (G-P Combo w/RJ; Corning, Hergiswil, Switzerland) on 5 g of whole soybean *meju* sample mixed with 5 mL of distilled water.

Amino-type nitrogen The analysis of amino-type nitrogen (12) was performed using the formalin titration method. After 80 mL of distilled water was added to 20 mL of pretreated solution, the solution was adjusted to pH 8.4 by adding 0.1 N NaOH. Subsequently, 20 mL of neutral formalin was added to the solution. The solution was titrated with 0.1 N NaOH to reach pH 8.4. The amount of amino-type nitrogen was calculated using the following equation:

$$\text{Amino-type nitrogen (\%)} = \frac{[\text{sample titration (mL)} - \text{blank test (mL)}] \times 1.4 \times F}{\text{sample (g)}} \times 100 \quad (3)$$

where the constant, 1.4, is the amount (mg) of amino-type nitrogen equal to 1 mL of 0.1 N NaOH.

Volatile organic acids For the analysis of volatile organic acids, each sample was diluted three-fold with pure water, and filtered with a membrane filter (0.45 μm). Subsequently, 0.3 μL of 2% H_2SO_4 was added to 5.7 mL of the filtrate, and 3 mL of the filtrate was injected into GC. The analysis conditions were as follows: instrument = Shimadzu GC 8A with FID, oven temperature = 150°C, column material = 10% PEG 6,000, injector temperature = 200°C, carrier gas = N_2 (40 mL/min), and column size = 3 mm × 1 m (stainless).

Nonvolatile organic acids Two hundred g of germinated *meju* was reflux extracted with 800 mL of ethanol at 85°C, filtrated, and vacuum-evaporated. Finally, 2 mL of 14% BF_3/methanol was added. The desalted sample was methylated by reacting at 80°C for 30 min. Methyl ester was then transferred into the chloroform layer by adding 4 mL of saturated ammonium sulfate and chloroform, and dehydrated by adding a small amount of anhydrous sodium sulfate. Subsequently, 0.5 L of the dehydrated solution was injected into GC and analyzed. The analysis conditions were as follows:
Instrument = DS 6200 (Donam System Inc., Korea), oven temperature = 60°C (1 min) - 10°C/min - 220°C (5 min), injector temperature = 230°C, carrier gas = N_2 (2 mL/min) and column = DB - Waxter (0.53 × 30 m).

Free amino acids The whole soybean *meju* was homogenized and extracted with 1% picric acid, and then passed through a Dowex 2×8 (Cl-form, 100-200 mesh) column filled with sodium citrate buffer (pH 2.2) to remove and evaporate the picric. The extract wasfiltered by a membrane filter (0.45 μm) and injected into an automatic amino acid analyzer (Biochrom 20; Uppsala, Sweden) and quantified. The analysis conditions were as follows: buffer flow rate = 20 mL/hr; ninhydrin flow rate = 20 mL/hr; temperature gradient = 35, 74, 80, and 37°C; wavelength decreased to 440 from 570 nm; column length = 46×250 nm; and injection volume = 20 μL.

Quantification of isoflavones by HPLC The analysis

of isoflavones was performed according to the method described by Wang *et al.* (13) with slight modifications (14). Here, 20 uL of filtrate was injected into HPLC equipped with a Bondapack C18 column after the system had been equilibrated at ambient temperature, and the UV detector stabilized with the mobile phase (methanol-1 mM ammonium acetate, 6:4) at a flow rate of 1 mL/min for 30 min. The effluent was detected at 254 nm and the chromatogram was recorded for 20 min. The isoflavones were identified by the retention times of the added standards, and their contents were calculated by comparing their peak areas with those of the standards.

Results and Discussion

Sprouting characteristics of the raw soybeans Figure 2A shows the germination rate results measured at 12-hr intervals from adding water for 2 hr after the soybeans had soaked for 4 hr, and plating them in a constant-temperature room controlled at 25°C. Visible changes were not observed until 12 hr of germination and the germination rate after 24 hr was 23.0±1.2%. The germination rate increased rapidly to 90.2±1.3% at 36 hr of germination and reached a level of 99.4±0.3% at 60 hr of germination. The total yield of the soybeans after soaking was 170±11% compared to the dried soybeans. As the germination process progressed, the total yield slowly increased and reached a level of 280±8% by the 60th hr, as described in Fig. 2B. There have been few reports so far related to the yield of bean sprouts according to different germination periods. The bean sprout yield by the 5th day of cultivation was high, and presented in the order of *Subaktae* (738%) > *Eunhakong* (705%) > *Seomoktae* (683%) (9). The yield of the bean sprouts was generally higher in small seeds than in large seeds (15). When the bean sprouts were cultivated by irrigating with 0.3 ppm of ozone water, they showed 10-17% higher yields than when they were irrigated with regular tap water (16). The root length changes of the soybeans during germination are shown in Fig. 2C, and the photographs are displayed in Fig. 2E-J. Visible changes in the soybeans did not appear until the 12th hr of germination. An embryo separated and began to grow by the 24th hr of germination, It then exhibited rapid growth reaching a length of 1.4±0.1 cm by the 36th hr, and a length of 4.1±0.2 cm at 60 hr of germination. These results are slightly faster than those reported by Kim *et al.* (17), in which *Dawonkong*, *Taekwangkong*, and *Myungjunamulkong* showed the root separating from the embryo after 34 hr of germination. It is believed these variations may have resulted from differences in the soaking times and germination environments. Kim *et al.* (18) reported that growth velocity of the sprout was inversely proportional to the weight of the raw soybean. The changes in the relative dry weights of the soybeans that occurred during germination are shown in Fig. 2D. At 12 hr after germination, the relative dry weight was 73.0±1.1% compared to the control, and it continued to gradually decrease with increasing germination time, reaching a level of 62.2±0.7% by 60 hr.

Figure 3 shows the changes in soybean isoflavone content that occurred during the germination process. The isoflavone content were in the order of genistein >

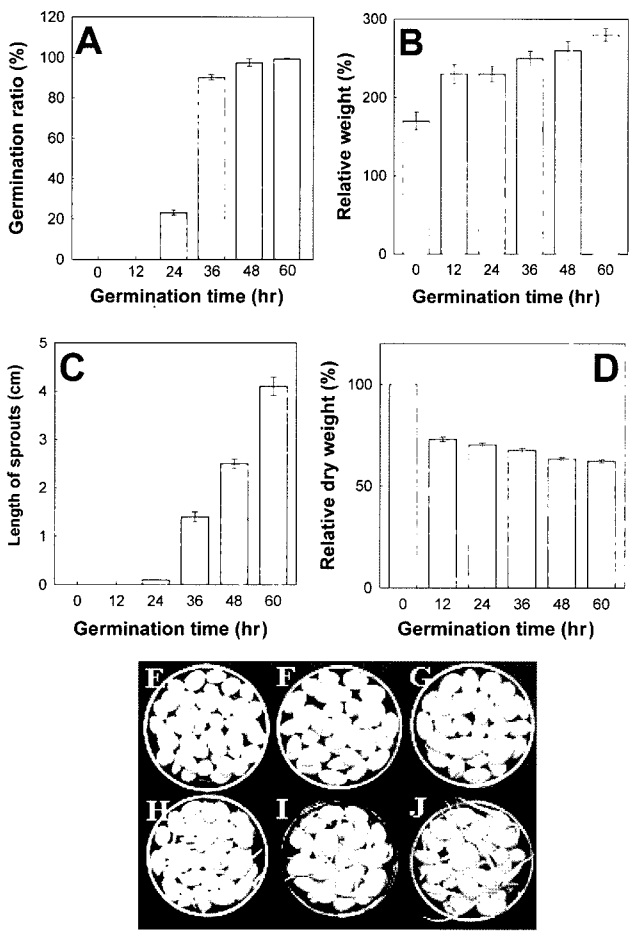

Fig. 2. Germination characteristics of soybeans. A, Germination ratio; B, relative weight; C, length of soybean sprouts; D, relative dry weight; E-J: The photo-graph of sprouted soybeans according to germination time. (E, soaked for 4 hr at 20°C; F, germinated for 12 hr after soaking; G, germinated for 24 hr after soaking; H, germinated for 36 hr after soaking; I, germinated for 48 hr after soaking; J, germinated for 60 hr after soaking).

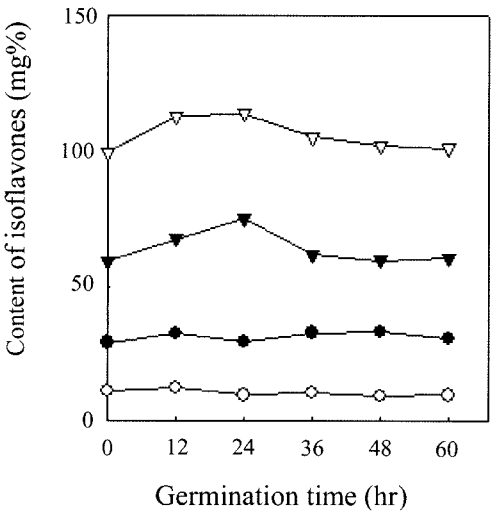

Fig. 3. Changes in isoflavone contents during soybean germination. –▽–, Total isoflavone; –▼–, genistein; –○–, glycitein; –●–, daidzein.

daidzein > glycitein, and remained in that order as germination progressed. It was confirmed that total isoflavone content immediately after soaking was 100.1 mg%, increasing in the beginning of the germination process; it continued increasing to 114.0 mg% by 24 hr of germination, but decreased thereafter. The isoflavone content at 60 hr of germination was 101.6 mg%.

Kim *et al.* (17) identified isoflavone content in *Myeongjunamulkong*, *Taekwangkong*, and *Dawonkong* soybeans based on the germination period and found that total isoflavone content reached a maximum level, increasing by 20-50% until 24 hr of germination. The aglycone types of daidzein and genistein, especially increased in this study, and these results agree with the results of our study. In addition, Lee *et al.* (6) reported that isoflavone content increased significantly as by producing soymilk from germinated beans, and the highest evaluated preference was for soymilk produced with beans that had germinated for 12 hr.

Color, pH, and amino-type nitrogen differences in whole soybean *meju* according to the degree of germination of the raw soybeans Table 1 shows the pH levels and colors of whole soybean *meju* fermented with germinated soybeans differing in germination times by increments of 12-hr. The pH level was between 6.3 and 6.5 regardless of the germination level of the raw material. The L value of the whole soybean *meju* fermented with the non-germinated raw material was 48.3 and did not show a large difference, ranging between the values of 46.0-48.3. The a value was 0.9 in the control, and the a value of the whole soybean *meju* increased as the germination time of the raw soybeans increased.

Consequently, the whole soybean *meju* produced with the beans germinated for 48 hr showed a b value of 3.2. The b value did not show a large difference according to the degree of germination of the raw material; also, the a/b values and ΔT (48.8-50.4) did not show much variation.

The results showed that the content of amino-type nitrogen in whole soybean *meju* fermented with non-germinated soybeans was 360.6, and it gradually decreased as the germination time of the raw material progressed. Consequently, the amino-type nitrogen content of the whole soybean *meju* fermented with soybeans that had germinated for 48 hr was only 261.5.

The organic acid differences of whole soybean *meju* according to the degree of germination of the raw soybeans The organic acid content of whole soybean *meju* differentiated according to soybean germination time are shown in Table 2. A total of 6 organic acids, including tartaric acid, malic acid, lactic acid, acetic acid, citric acid, and succinic acid were detected, and total organic acid content ranged from 963.1-1,145.3 mg%. Differences based on the degree of germination in the raw material were insignificant. As the germination of the raw material increased, the acetic acid content also increased. On the other hand, the citric acid content slightly decreased with increasing germination of the raw material. Studies relating to the organic acid content of general *meju* are abundant. Lee *et al.* (19) reported that the organic acid contents of *meju* produced using 4 species of beans, which are the recommended bean varieties for *meju* manufacture or soybean paste processing, were 2,300-2,900 mg%. Im *et al.* (20) stated that the non-volatile organic acid content of *meju* produced by the traditional method and an improved method were 739.9 and 1,153.3 mg%, respectively. Kim *et al.* (21) reported that the content of organic acids in *meju* fermented for 45 hr using *Aspergillus* sp. ranged from 2.77-5.94%, and differences in the content of the taste components were caused by a number of complex factors such as the types of microorganisms, fermentation environment, and the state of the raw materials. No studies regarding the organic acid content of *meju* produced with germinated beans have been reported previously. Thus, further studies must be conducted to investigate the optimum conditions for *meju* that is fermented with germinated soybeans, such as diversifying the variety of the raw soybeans used, the germination temperature of the raw soybeans, the fermentation conditions, and the species of microorganisms.

The free amino acid differences of whole soybean *mejus* according to the degree of germination of the raw soybeans The free amino acid composition levels of whole soybean *meju* according to the degree of germination of the raw soybeans are shown in Table 3. The level of free amino acids in the whole soybean *meju* made from non-germinated soybeans was 2,580.9 mg%. The free amino

Table 1. Color and pH of *meju* according to the germination time of raw soybeans

		Germination time (hr)				
		0	12	24	36	48
pH		6.5	6.3	6.3	6.3	6.3
ATN[1](mg%)		360.6	346.5	312.9	280.7	261.5
Color	L	48.3	47.7	46.0	47.0	46.5
	a	0.9	1.1	1.7	2.2	3.2
	b	13.9	16.3	16.2	15.6	15.9
	a/b	0.1	0.1	0.1	0.1	0.2
	ΔT[2]	50.3	50.4	48.8	49.6	49.1

[1]Amino-type nitrogen. [2]$\Delta T=(L^2+a^2+b^2)$.

Table 2. Composition of organic acids in *meju* according to the germination time of raw soybeans (mg%, dry weight)

Organic acid	Germination time (hr)				
	0	12	24	36	48
Tartaric acid	119.9	128.7	180.2	152.5	147.2
Malic acid	20.0	24.2	44.3	40.7	42.6
Lactic acid	348.2	269.9	274.7	296.1	283.9
Acetic acid	85.3	178.7	211.9	315.2	451.7
Citric acid	348.6	313.1	307.9	223.7	207.5
Succinic acid	74.1	48.5	48.3	22.5	12.4
Total	996.0	963.1	1,067.2	1,050.6	1,145.3

Table 3. Composition of amino acids in *meju* according to the germination time of raw soybeans (mg %, dry weight)

	Amino acid	Germination time (hr)		
		0	24	48
Sweet taste	Thr	104.0	98.3	111.9
	Ser	132.3	124.1	140.1
	Gly	116.8	111.7	113.0
	Ala	108.8	107.7	105.0
	Lys	255.4	248.6	240.0
	subtotal	717.3	690.4	710.1
Savory taste	Asp	229.5	251.8	291.1
	Glu	546.6	532.6	460.7
	Cys	39.5	26.5	37.2
	subtotal	815.6	810.9	789.0
Bitter taste	Met	38.0	36.5	40.3
	Ile	118.7	121.6	120.3
	Leu	224.1	216.1	173.9
	subtotal	380.8	374.2	334.5
Others	Pro	189.4	169.5	176.6
	Val	130.0	133.4	123.2
	Tyr	103.3	100.8	123.2
	Phe	147.6	143.2	172.8
	His	76.7	72.5	69.1
	Arg	20.2	18.7	21.2
	subtotal	667.2	638.1	686.1
Total		2,580.9	2,513.6	2,519.7

acid content of the whole soybean *meju* produced from soybeans that underwent 48 hr of germination was 2,519.7 mg%, which, according to the degree of germination, was an insignificant difference. The content of glutamic acid, a savory taste component, was the highest (460.7-546.6 mg%), followed by aspartic acid (229.5-291.1 mg%), lysine (240.0-255.4 mg%), leucine (173.9-224.1 mg%), and proline (169.5-189.4 mg%). The content of glutamic acid accounted for 21.2% of the total free amino acid content in the control and in the *meju* produced with beans germinated for 24 hr. In *meju* produced with beans germinated for 48 hr, the content of glutamic acid accounted for 18.1% of the total free amino acid content (data not shown). Among the free amino acids, threonine, serine, glycine, alanine, and lysine represent a sweet taste; aspartic acid, glutamic acid, and cystein represent a savory taste; and methionine, isoleucine, and leucine represent bitter tastes (22). Weak differences between the sweet taste components (690.4-717.3 mg%), savory taste components (789.0-815.6 mg%), and bitter taste components (334.5-380.8 mg%) were discovered according to the germination of the raw soybeans.

The isoflavone content differences in whole soybean *meju* according to the degree of germination of the raw soybeans The isoflavone content of whole soybean *meju* according to varying degrees of germination are shown in Table 4. The level of isoflavones in the whole soybean *meju* made from non-germinated soybeans was 124.5 mg% after 48 hr of fermentation. On the other hand, the isoflavone levels of whole soybean *meju* produced from soybeans germinated for 12 and 24 hr were 123.9 and 123.3 mg%, respectively. The isoflavone content of the *meju* ranged 123.0-124.5 mg%. The level of genistein was the highest followed by daidzein and glycitein throughout all the fermentation periods. Isoflavone content and composition varied greatly among the soybean sprouts, immature soybeans, and mature soybeans (3). Zhu *et al.* (23) investigated the effect of germination on the isoflavone content of two soybean varieties and reported that maximum isoflavone content can be controlled by the degree of germination of the soybean seeds. Finally, Kim *et al.* (24) reported that total isoflavone content increased by 13% during the initial germination period (6-24 hr), and decreased thereafter.

Table 4. Composition of isoflavones in *meju* according to the germination time of raw soybeans (mg%)

Isoflavone	Germination time (hr)				
	0	12	24	36	48
Daidzein	41.9	40.7	41.0	40.2	44.5
Glycitein	6.8	5.7	5.9	5.9	6.1
Genistein	75.1	77.5	76.4	76.9	73.9
Total	123.8	123.9	123.3	123.0	124.5

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