

Changes of Isoflavones and Fatty Acids in *Eoyukjang*, a Traditional Korean Fermented Soysauce Prepared under the Ground in a Pot or an Incubator

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Abstract *Eoyukjang* is a traditional Korean sauce made of cooked soypaste or *meju* supplemented with fish and meats at least 1 year of fermentation period. *Eoyukjang* was recovered according to the traditional method and stored under the ground in a pot without plastic packaging (13G: 13 month fermentation under the ground) or in an incubator wrapped with plastic packaging (6I, 12I, and 18I: 6, 12, and 18 month fermentation, respectively). Distribution of isoflavones and fatty acids were monitored by high performance liquid chromatography (HPLC) and gas chromatography (GC), respectively. Total isoflavones in 13G, 6I, 12I, and 18I were 3.792, 0.387, 0.460, and 0.510 $\mu\text{mol/g}$, respectively. Samples of 13G had at least 8.24 times higher isoflavone contents than samples from 12I. Aglycones were the major isoflavones in *eoyukjang* and were found more than 92% in 13G and 39-63% in incubated samples. In fatty acid analysis, the ratios of unsaturated to saturated fatty acids from 13G were higher than those from 6I, 12I, and 18I. Traditional fermentation methods using a pot may allow more migration of air and moisture than samples wrapped with plastic packaging, which caused the difference in the distribution of isoflavones and fatty acids.

Keywords: *eoyukjang*, isoflavone, fatty acid, preparation condition

Introduction

Soybean is an important food ingredient in Korean food cultures and many soy-based fermented foods have been developed and consumed including *meju*, *doenjang*, *kochujang*, or *cheonggukjang*. Traditional *jang* typed foods such as *doenjang* and *cheonggukjang* use cooked soybeans with fermentation of diverse microorganisms. *Doenjang* is a soypaste fermented mainly with *Aspergillus* species with a relatively long fermentation time and *cheonggukjang* is made of cooked soybeans fermented with mainly *Bacillus subtilis* or *Bacillus natto* within 1-2 days (1-3). These soy fermented foods have been excellent nutritional sources of essential amino acids and essential fatty acids to people who do not consume enough animal proteins and lipids.

Eoyukjang is a traditional Korean sauce used for dressing and dipping and made of cooked soypaste or *meju*, supplemented with fish and meat. Historically, *eoyukjang* has been consumed among the noble society due to the expensive ingredients and long fermentation period. These kinds of the Noble's foods were called as 'Banga foods' and consumed in the capital city, Seoul since the Joseon Dynasty (A.D. 1392-1910). *Eoyukjang* has characteristic odor and taste, which can not be found in *doenjang*, *meju*, and *cheonggukjang* (4-6). Characteristic flavor of *eoyukjang* may be derived from enzymatic activities in raw ingredients and salt-tolerant microorganisms during a long fermentation.

Recently, consumers' need for the traditional fermented soy foods has increased because of the high awareness of health beneficial functions in foods and of traditional food cultures.

Consumption of soy-based foods is increasing in both Oriental and Western countries partly due to the epidemiological results showing positive correlations of soy consumption and decreasing the risks of cardiovascular disease and of various cancers (7,8). Isoflavones are phytoestrogenic compounds in soybeans and regarded as one of major bioactive components in soy-based foods. Isoflavones in soybeans are found in 3 chemical structures including aglycones (genistein, daidzein, glycitein), and their corresponding β -glucosides, acetyl- β -glucosides, and malonyl- β -glucosides (9). β -Glucosides or aglycones are major chemical forms of isoflavones in *jang* typed foods due to the β -glucosidase activity in microorganisms and conventional cooking process depending on the fermentation conditions (3,10,11).

Soybeans contain not only about 0.1-0.4% isoflavones but also 20% fat. Because soybean is major ingredient in *meju*, profiles of major fatty acids in traditional *jang* typed foods should be similar to those of soybean (12,13). Fat contents and profiles of fatty acids in *eoyukjang* can affect not only the oxidative stability but also the sensory quality. High fat content may influence the consumers' acceptance when body weight is concerned. Also, fatty acid compositions could be useful markers to monitor the ingredients especially fish and red meats or to discriminate *eoyukjang* with other mimic foods.

Systematic studies on *eoyukjang* are rare in literature and some previous studies focused mainly on the identification

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of volatiles or volatile changes with other samples. Yoon *et al.* (4) identified 36 volatile components in *eoyukjang* and butanoic acid and methional were found as major aroma-active volatiles. Lim *et al.* (5) showed that volatile components of *eoyukjang* and commercially available soy sauces were discriminated through electronic nose based on gas chromatography (GC) with surface acoustic wave sensors. However, isoflavone changes and distribution of fatty acids during *eoyukjang* preparation are not reported in the literature.

Traditional way for *eoyukjang* preparation needs more than one year of fermentation period in a pot under the ground. To produce reproducible qualities of *eoyukjang*, fermentation conditions of samples should be controlled properly using incubators. Therefore, it is necessary to compare the difference of qualities between traditionally prepared and incubated *eoyukjang* samples.

The objective of this study was to monitor the profile changes of isoflavones and fatty acid during *eoyukjang* preparation with different storage conditions.

Materials and Methods

Materials Soybeans, fish, and red meats were purchased from local grocery markets (Seoul, Korea). Mixtures of standard fatty acids, boron trifluoride solution (14% methanolic), daidzein, and genistein were purchased from Sigma-Aldrich (St. Louis, MO, USA), and 10 standard compounds including glycitein, daidzin, genistin, glycitin, acetyl- β -daidzin, acetyl- β -genistin, acetyl- β -glycitin, malonyl- β -daidzin, malonyl- β -genistin, and malonyl- β -glycitin were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). High performance liquid chromatography (HPLC)-grade methanol and acetic acid were purchased from Fisher Scientific Ltd. (Fairlawn, NJ, USA).

Sample preparation Raw soybeans were cooked and prepared for the preparation of *meju*, which is a traditional Korean soypaste used as a main ingredient for *doenjang* or

kochojang. *Meju* was mixed with various fish and meats according to the ratio shown in Table 1. Two types of samples were prepared. Total weight of 1 sample was 1,042 kg and stored under the ground in a pot without plastic packaging. The other set of samples were wrapped with plastic packaging and stored in an incubator. The weight of each sample in the incubator was 43.2 kg. The temperature of an incubator was programmed according to the temperature of the underground: 3°C for 4 months, 13°C for 2 months, 14°C for 4 months, 4°C for 2 months, 3°C for 4 months, and 13°C for 2 months. Samples were prepared at the year of 2006, February. Sample stored under the ground was taken at 13 month old, designated as 13G, and samples from the incubator were measured at 6, 12, and 18 month old, which were designated as 6I, 12I, and 18I, respectively. At each sampling time, 2 different bags of incubated samples were prepared. After sampling, *eoyukjang* was centrifuges and solid matrix was freeze-dried and used for the further analysis.

Isoflavone analysis Isoflavone analysis method was adapted from Yang *et al.* (3). Briefly, 12 mL solvent mixture was added to 1 g of sample and mixed for 2 hr. After centrifugation at 2,208 \times g for 10 min (Hanil, Incheon, Korea), aliquot of 1 mL of supernatant was taken and dried under nitrogen gas flow. One mL of methanol was added and the mixture was filtered with a 0.2- μ m syringe filter (Alltech Associates Inc., Deerfield, IL, USA) and then injected to HPLC (Hitachi, Tokyo, Japan).

Isoflavones were separated using a HPLC (Hitachi, Tokyo, Japan) equipped with an ultraviolet detector, a 4- μ m Waters Novapak C₁₈ reversed-phase HPLC column (150 \times 3.9 mm i.d.), a Novapak C₁₈ guard column, and a 0.5- μ m pre-column filter. HPLC condition for isoflavone analysis and quantification of each isoflavone were adapted from the results of Yang *et al.* (3). All the analyses were conducted triplicate.

Fatty acid analysis Lipid in samples was extracted using *n*-hexane in a Soxhlet apparatus. Fatty acid methyl ester

Table 1. Ingredients used for preparation of *eoyukjang*

Ingredients	Samples stored in an incubator (for I sample)		Samples stored under the ground (for 13G sample)	
	Contents	Relative (%)	Contents	Relative (%)
<i>Meju</i>	320 g	7.40	7,700 g	7.39
Beef	110 g	2.54	2,700 g	2.59
Chicken	125 g	2.89	3,000 g	2.88
Pheasant meat	60 g	1.39	1,400 g	1.34
Gray mullet	75 g	1.73	1,700 g	1.63
Red sea bream	115 g	2.66	2,700 g	2.59
Mussel	35 g	0.81	800 g	0.77
Spiny-lobster	45 g	1.04	1,000 g	0.96
Abalone	5 g	0.12	110 g	0.11
Garlic	20 g	0.46	450 g	0.43
Spring onion	20 g	0.46	400 g	0.38
Ginger	20 g	0.46	425 g	0.41
NaCl solution (36.4%, w/v)	2.5 L	57.80	60 L	57.56
Additional NaCl	875 g	20.23	21,850 g	20.97

Table 2. Distribution of isoflavone ($\mu\text{mol/g}$) during *eoyukjang* preparation

Sample ¹⁾	Raw	6I	12I	18I	13G
DE	0.110 \pm 0.004 ²⁾	0.166 \pm 0.004	0.200 \pm 0.019	0.180 \pm 0.054	1.140 \pm 0.168
DI	0.440 \pm 0.034	0.039 \pm 0.002	ND ³⁾	0.103 \pm 0.005	0.061 \pm 0.014
ADI	0.080 \pm 0.003	ND	0.014 \pm 0.002	ND	0.084 \pm 0.006
MDI	1.241 \pm 0.052	0.040 \pm 0.002	0.015 \pm 0.001	ND	0.012 \pm 0.001
GE	0.159 \pm 0.003	0.031 \pm 0.009	0.007 \pm 0.001	0.019 \pm 0.005	2.011 \pm 0.322
GI	0.378 \pm 0.024	0.054 \pm 0.002	0.033 \pm 0.003	0.055 \pm 0.003	0.006 \pm 0.000
AGI	0.026 \pm 0.000	0.018 \pm 0.000	0.020 \pm 0.000	0.023 \pm 0.002	0.018 \pm 0.001
MGI	4.013 \pm 0.070	ND	ND	ND	0.033 \pm 0.005
GY	0.015 \pm 0.002	0.034 \pm 0.001	0.086 \pm 0.008	0.005 \pm 0.000	0.376 \pm 0.051
GYI	0.109 \pm 0.030	ND	0.071 \pm 0.019	0.085 \pm 0.004	ND
AGYI	ND	ND	0.010 \pm 0.003	0.019 \pm 0.003	0.005 \pm 0.000
MGYI	0.053 \pm 0.014	0.004 \pm 0.000	0.006 \pm 0.001	0.022 \pm 0.001	0.045 \pm 0.003
TI	6.624 \pm 0.205e ⁴⁾	0.387 \pm 0.021 ^a	0.460 \pm 0.048 ^b	0.510 \pm 0.057 ^c	3.792 \pm 0.550 ^d

¹⁾DE, daidzein; DI, daidzin; ADI, acetyl- β -daidzin; MDI, malonyl- β -daidzin; GE, genistein; GI, genistin; AGI, acetyl- β -genistin; MGI, malonyl- β -genistin; GY, glycitein; GYI, glycitin; AGYI, acetyl- β -glycitin; MGYI, malonyl- β -glycitin; TI, total isoflavones.

²⁾Mean \pm SD ($n=6$ for incubated samples and $n=3$ for 13G and raw soybeans)

³⁾Not detected.

⁴⁾Different letters are significant in the same row at $p<0.05$.

Table 3. Relative concentration of isoflavones in *eoyukjang*

Sample	Aglycone (%)	β -Glucoside (%)	Acetyl- β -glucoside (%)	Malonyl- β -glucoside (%)
Raw soybean	4.305 \pm 0.015 ^{a1)}	13.965 \pm 0.924 ^b	1.606 \pm 0.008 ^a	80.124 \pm 0.912 ^d
6I	59.820 \pm 0.105 ^c	24.085 \pm 0.101 ^c	4.714 \pm 0.001 ^c	11.382 \pm 0.016 ^c
12I	63.680 \pm 2.292 ^d	22.440 \pm 2.579 ^c	9.409 \pm 1.026 ^d	4.471 \pm 0.192 ^b
18I	39.478 \pm 7.129 ^b	48.060 \pm 6.586 ^d	8.124 \pm 0.053 ^d	4.338 \pm 0.596 ^b
13G	92.969 \pm 0.806 ^{e2)}	1.756 \pm 0.332 ^a	2.858 \pm 0.278 ^b	2.417 \pm 0.401 ^a

¹⁾Mean \pm SD ($n=6$ for incubated samples and $n=3$ for 13G and raw soybeans); Different letters are significant in the same column at $p<0.05$.

(FAME) was prepared by a modified method using BF_3/MeOH (14% boron trifluoride) according to the AOAC 969.33 (14). All the analyses were conducted triplicate.

Gas chromatography (GC) Hewlett-Packard 5890-II gas chromatograph equipped with a flame ionization detector (FID), and a DB-23 (60 m \times 0.32 mm i.d., 0.25- μm film) from J&W Scientific (Folsom, CA, USA) was used for fatty acid analysis. The oven temperature was held at 100 $^\circ\text{C}$ for 1 min, increased from 100 to 195 $^\circ\text{C}$ at 15 $^\circ\text{C}/\text{min}$, from 195 to 210 $^\circ\text{C}$ at 1 $^\circ\text{C}/\text{min}$, and from 210 to 240 $^\circ\text{C}$ at 5 $^\circ\text{C}/\text{min}$, and held at 240 $^\circ\text{C}$ for 7.5 min. The temperatures of both injector and detector were 260 $^\circ\text{C}$. The flow rate of helium carrier gas was 1.1 mL/min, and the split ratio was 1:50.

Statistical analysis Data were analyzed statistically by analysis of variance (ANOVA) and Duncan's multiple-range test using SPSS software program (SPSS Inc., Chicago, IL, USA). A p value <0.05 was considered significant.

Results and Discussion

Distribution of isoflavones in *eoyukjang* Profile changes of isoflavones during *eoyukjang* preparation are shown in Table 2. Total isoflavones (TI) in raw soybean were 6.624 $\mu\text{mol/g}$ while TI from 6I, 12I, and 18I were 0.387, 0.460, and 0.510 $\mu\text{mol/g}$, respectively. Relative percentage of *meju* among total weight was about 7.40% and about 0.490

$\mu\text{mol/g}$ ($=6.624 \times 0.074$) of TI was expected in *eoyukjang*. Usually, soaking and heating process causes some loss in TI of soybeans (9,15). TI from 12I and 18I were significantly higher than 6I ($p<0.05$). 12I and 18I endured longer incubation than 6I, which may affect the profiles and concentration of isoflavones in *eoyukjang*. However, TI from 13G was 3.792 $\mu\text{mol/g}$, which was significantly higher concentration than those of all the incubated samples ($p<0.05$). 13G contained 8.24 times higher isoflavone concentration compared to TI from 12I.

The differences of fermentation conditions among incubated and ground stored samples may come from various factors including the presence or absence of packaging materials, fermentation temperature, and/or fermentation microorganisms. One of major factors could be wrapping process with plastic packaging materials, which could impede the migration of air and moisture. On the contrary, samples without packaging might have more chance for the migration of air and moisture, and different fermentation could be performed and ingredients were concentrated due to the dehydration. We observed large amount of salt precipitation outside of the pot containing *eoyukjang*. The pot or '*onggi*' jar was made of clay with high temperature flame and migration of air and moisture in a pot is well-known phenomenon. Seo *et al.* (16) showed that *onggi* jar is composed of 53-161 nm sized porous matrix depending on the glazing condition, the size of which was big enough for the migration of air, minerals,

Table 4. Distribution of major fatty acids (%) in eoyukjang

Fatty acids ¹⁾	Raw soybean	6I	12I	18I	13G
C14:0	-	0.77±0.33 ²⁾	1.40±0.10	1.17±0.15	-
C16:0	9.71±0.10	14.56±0.03	17.81±1.10	15.90±1.31	9.56±0.57
C16:1	-	2.37±0.06	2.90±0.06	2.71±0.20	0.23±0.01
C18:0	2.93±0.05	4.76±0.11	5.66±0.41	5.11±0.64	3.08±0.41
C18:1 (<i>trans n-9</i>)	-	0.12±0.03	0.22±0.08	0.36±0.14	0.05±0.00
C18:1 (<i>cis n-9</i>)	27.26±0.12	28.78±0.26	32.32±1.68	32.58±1.39	26.32±0.92
C18:1 (<i>n-7</i>)	-	1.91±0.04	2.49±0.04	2.20±0.02	1.64±0.20
C18:2 (<i>n-6</i>)	50.27±1.10	37.42±0.35	29.08±2.31	30.75±3.23	50.41±1.10
C18:3 (<i>n-3</i>)	7.98±0.20	5.15±0.09	3.56±0.42	3.90±0.42	7.23±0.25
C18:2, CLA	-	0.10±0.06	0.07±0.01	0.04±0.00	-
C20:1	0.22±0.01	0.78±0.01	1.25±0.04	0.97±0.03	0.25±0.00
C22:0	1.02±0.01	0.38±0.01	0.40±0.04	0.29±0.03	0.50±0.02
C20:5 (<i>n-3</i>), EPA	-	0.49±0.01	0.46±0.04	0.75±0.09	-
C22:1	-	0.16±0.06	0.38±0.16	0.25±0.05	-
C24:0	-	0.18±0.08	0.18±0.05	0.12±0.01	-
C24:1	-	0.22±0.09	0.19±0.03	0.38±0.04	-
C22:6 (<i>n-3</i>), DHA	-	0.80±0.02	0.50±0.19	1.20±0.13	0.17±0.00
Unsaturated Saturated fatty acids (U/S)	6.00	3.82	2.84	3.36	6.37

¹⁾CLA, conjugated linoleic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

²⁾Mean ±SD (*n*=6 for incubated samples and *n*=3 for 13G and raw soybeans).

and salts. The authors also showed that the permeance of oxygen and carbon dioxide through *onggi* jar decreased after soy sauce fermentation. Therefore, increases of TI may be due to the concentration effects of moisture migration to outside and more solid matrix was remained in 13G samples. However, content of moisture in samples were not determined in this study.

Relative percentages of isoflavones during preparation of *eoyukjang* are shown in Table 3. Malonyl derivatives were the major isoflavones in raw soybeans and aglycones were detected less than 5%. Aglycones were the main isoflavones in 6I, 12I, and 18I samples with the range from 39.478 to 63.680% and β -glucosides ranges from 22.440 to 48.060%, which implies that β -glucosidase was active during *eoyukjang* preparation. As fermentation period increased from 6I to 18I, malonyl derivatives decreased.

Significant changes of aglycones and β -glucosides were observed in 13G compared to those in incubated samples (*p*<0.05). Relative percentage of aglycones and β -glucosides in 13G was 92.969 and 1.756%, respectively, which implies that more active fermentation occurred in 13G due to the environmental factors.

Generally, fermented soy foods with extended period such as *doenjang* contained more aglycones while short period fermented soy foods like *cheonggukjang* had less aglycones and more β -glucosides depending on the fermentation conditions. Kim and Yoon (10) reported that aglycones were major isoflavone forms in *doenjang*. Jang *et al.* (11) analyzed isoflavone contents in *cheonggukjang* and showed that major isoflavone forms were aglycones while Yang *et al.* (3) reported *cheonggukjang* had more β -glucosides than aglycones.

Distribution of fatty acids in eoyukjang Profile changes of fatty acids during *eoyukjang* preparation are shown in

Table 4. Compared to soybeans, new fatty acids including 14:0, 16:1, 9 *trans* 18:1, omega-7 18:1, conjugated linoleic acid (CLA), 20:1, 20:5 (eicosapentaenoic acid, EPA), 22:1, 24:0, 24:1, and 22:6 (docosahexaenoic acid, DHA) were detected in *eoyukjang*, which comes from fish and meats. The number of fatty acids from 13G was less than those from 6I, 12I, and 18I. The ratios of unsaturated to saturated fatty acids (U/S) from 13G were higher than those from 6I, 12I, and 18I. Concentrations of highly unsaturated fatty acids such as EPA and DHA in 13G were lower than those of 6I, 12I, and 18I. This difference may come from enzymatic activities from ingredients and microorganisms, and chemical reactions such as lipid oxidation among ingredients.

The changes of isoflavones or fatty acid compositions during preparation of fermented foods have been reported in the literature. *Doenjang* is a fermented soy paste and some manufactures produce more than 1 year old *doenjang*. Kang and Lee (12) compared lipid contents and fatty acid compositions in traditionally prepared *doenjang* and *koji* added *doenjang*. The authors reported contents of total lipid and free fatty acids changed in *doenjang* as fermentation period prolonged while uniform trends of fatty acid compositions were not observed in traditionally prepared *doenjang*. Fatty acid composition of 15 traditional *doenjang* was analyzed by Park *et al.* (13). They reported that *doenjang* contained linoleic (52.2%), oleic (20.7%), and linolenic acids (8.7%) as major unsaturated fatty acids. Park *et al.* (17) found out the presence of arachidonic acid, linoleic acid, and linolenic acid in 90 day old *doenjang* and distribution of fatty acids were not consistent during fermentation. Other traditional fermented soy foods like soy sauce and *kochujang* did not follow the fatty acid profiles of raw soybeans because of the different processing and additional ingredients. Soy sauce, which is made from

soluble compounds from *meju*, contained relatively high amount of palmitic, stearic, and myristic acids in decreasing order and low concentration of linoleic acid (18), which may be due to the biotransformation of microorganisms during fermentation and removal of solid matrix. Chun *et al.* (19) analyzed fatty acid profiles in *kochujang* and showed high contents of oleic acid and low amount of linoleic acid during preparation. *Eoyukjang* had different fatty acid profiles from cooked soybeans due to the presence of other ingredients such as fish and meats (Table 4).

Migration of air and moisture through containers may play an important role on the degree of fermentation, distribution of ingredients, and sensory quality of *eoyukjang*. This study first showed that containers like a pot or *onggi* jar with proper permeance of gas and moisture were needed to recover and to produce *eoyukjang* in the industrial scale.

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