Changes in isoflavone composition of soya seeds, soya curd and soya paste at different processing conditions

ILL-MIN CHUNG – SU-HYUN SEO – INMYOUNG PARK – JOUNG-KUK AHN – EUN-HYE KIM – MIN-YOUNG KIM – SEUNG-HYUN KIM

Summary

The present study describes the effects of processing, fermentation and aging on the total and individual isoflavone contents in 20 cultivars of soya (seeds) as well as soya curd (tofu) and soya paste (chunggukjang) made from each soya cultivar. Mean total isoflavone contents were 12.03 $mmol \cdot kg^{-1}$ in seeds, 5.94 $mmol \cdot kg^{-1}$ in tofu, 12.38 $mmol \cdot kg^{-1}$ in chunggukjang aged for 3 days, and 12.75 $mmol \cdot kg^{-1}$ in chunggukjang aged for 6 days. Recovery rates of total isoflavones were 103–106% for chunggukjang, but only ~50% for tofu. Total or individual isoflavone profiles differed between seeds, tofu and chunggukjang. In the present study, the group effect ("paste/curd" versus "cooked with rice") of total isoflavones (percentage, mole basis) was observed in chunggukjang aged for 3 and 6 days ($P < 0.01$) and not for soya seeds or tofu. In addition, chunggukjang contained larger amounts of isoflavone aglucons, in particular daidzein, compared to soya seeds or tofu.

Keywords

isoflavone profile; soya cultivar; soya food; fermentation

The consumption of soya products has many health benefits, including reducing the occurrence of obesity, metabolic disorders [1, 2] and menopausal symptoms, protection against osteoporosis [3–5] and growth inhibition of human breast or prostate cancer [6–8]. Many health benefits of soya products are derived from the contained isoflavones.

The contents and profiles of isoflavones in soya seeds or soya-based foods are influenced by the genetic background (i.e. soya cultivar), growth environment (i.e. cropping location, method, year) [9–11] and processing conditions, such as pH, heating, soaking, deforming, pressure or application of endogenous/exogenous β–glucosidases [12–16]. Raw soya seeds usually contain 0.3–0.8% (dry basis) isoflavones, malonyldaidzin and malonylgenistin being the major forms [17]. During food processing, 15–20% of the total isoflavones are lost in the residues of soya curd, 30–31% in whey, 44% during coagulation of tofu, or 49% during heating in tempeh [14, 18]. Furthermore, about 30% of the total isoflavones (mole mass) were shown to be lost during soymilk production [14]. WANG and MURPHY [15] reported that raw (dry, non-cooked) soya curd contained 532 mg isoflavones per kilogram. FRANKE et al. [19] reported that raw soya curd and cooked soya curd contained 297 mg and 258 mg isoflavones per kilogram, respectively. The differences in isoflavone contents and profiles in soya curds were attributed to either the use of different soya cultivars, different processing procedures, or both [15, 19].

Asian countries commonly consume soya in the form of soya paste, soya milk, soya curd or soya bread. These soya-based products undergo different treatment, so they contain different amounts isoflavones with different profiles. Chunggukjang, the soya paste produced by fermentation with *Bacillus subtilis* or *Bacillus natto*, is similar

Ill-Min Chung, Su-Hyun Seo, Joung-Kuk Ahn, Eun-Hye Kim, Min-Young Kim, Department of Applied Life Science, College of Life and Environmental Science, Konkuk University, Seoul 143-701, Korea.
Inmyoung Park, Department of Agricultural Biotechnology and Center for Agricultural Biomaterials, Seoul National University, Seoul 151-921, Korea.
Seung-Hyun Kim, Center for Analytical Chemistry, Division of Metrology for Quality of Life, Korea Research Institute of Standards and Science, P. O. Box 102, Yuseong, Dajeon 305-600, Korea.

Correspondence author:
Seung-Hyun Kim, tel.: +82-42-868-5939, fax: +82-42-868-5800, e-mail: seunghyun.kim@kriss.re.kr

to *natto* of Japan, *shuidouchi* of China, *thua nao* of Thailand or *kinema* of India Nepal or India. Chunggukjang contains various essential amino acids, isoflavones, saponin, trypsin inhibitors and tocopherols [20, 21]. This food product has raised considerable interest due to reports of its ability to inhibit angiotensin-converting enzyme (ACE), reduce blood pressure, increase the body's ability to dissolve blood clots and to improve blood circulation [21]. However, nutritive composition of soya curd (tofu) is somewhat differed from the fermented soya-based foods. Soya curd contains less nutritive components compared to fermented soya-based foods due to the big loss of nutritive components during processing (filtering, solidification/coagulation, salting). Also, soya curd contains large amounts of β-glycoside isoflavones, whereas fermented soya-based foods contain more aglycone isoflavones. Furthermore, soya curd and fermented soya-based foods contain different major phenolics [13, 14, 16, 22].

Several studies reported the effects of processing conditions on isoflavone contents and profiles of some soya-based foods like tofu and soya milk. However, information on isoflavones in tofu and chunggukjang, when produced from defined soya cultivars, are scarce. Therefore, the present study investigated the effects of fermentation and aging on the total and individual isoflavones contents of 20 chunggukjang samples, each produced from a single soya cultivar. These chunggukjang samples were aged for 3 or 6 days, since aging is known to be an important factor in terms of the quality of chunggukjang. The present study also investigated the change of isoflavone contents and profiles of tofu produced from identical 20 soya cultivars.

MATERIALS AND METHODS

Sample preparation: soya cultivation, tofu (soya curd) and chunggukjang (soya paste)

Twenty soya cultivars were grown in the northern part of Gyeonggi province, Korea, in 2008. The experimental layout, climatic conditions and plot management were as previously described [23]. In the present study, soya cultivars were classified into "paste and curd (P/C)" group or "cooked with rice (C/R)" group. Each group consisted of ten soya cultivars. Briefly, cultivars in the "P/C" group had high contents of proteins, yellowish seed coat and a high yield potential in the field (g·m^{-2}). Cultivars in the "C/R" group had larger seed size, swelled with soft seed texture after being soaked in the water, contained high amounts of reducing saccharides and had high levels of alkali-digestive value. To the best of our knowledge, these criteria were used as basic information for breeding of soya cultivars in Korea. The complete description of the soya cultivars were previously reported [22]. Seeds from 20 soya plants in each plot were harvested for each soya cultivar and stored at dehumidified room at 25–30 °C for 1 week. Soya seeds were selected by random, lyophilized at –47 °C, pulverized and stored (< 1 week) in a desiccator until analysis.

Tofu (soya curd) and chunggukjang (soya paste) were made on a laboratory scale (Fig. 1). Detailed data on processes for the production of tofu and chunggukjang were previously reported [22]. Because commercial chunggukjang usually ages for 2 – 3 days, we tested chunggukjang aging for 6 days in order to understand the effect of aging on isoflavone contents and profiles. Each tofu and chunggukjang was made in duplicate, lyophilized at – 47 °C, pulverized and stored (< 1 week) in a desiccator until analysis.

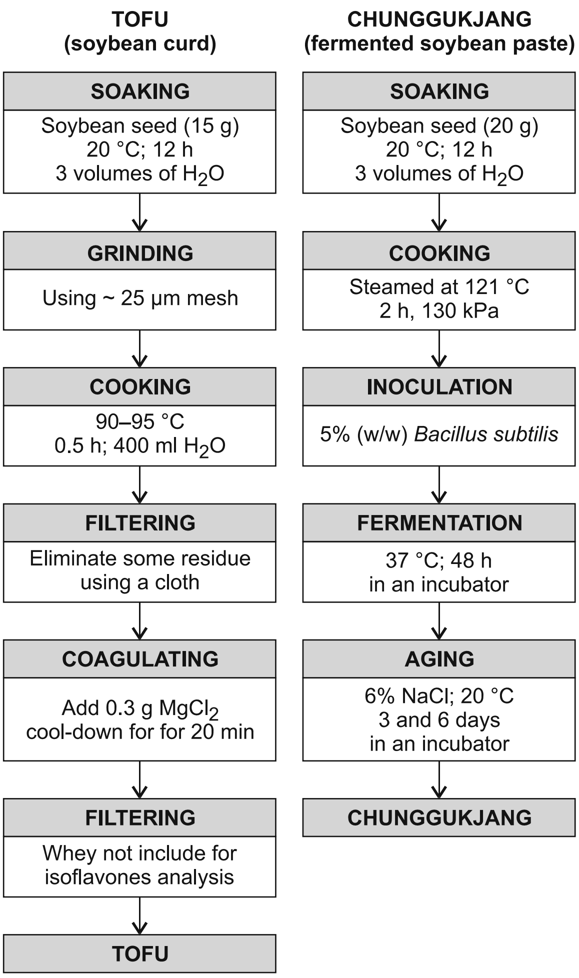

Fig. 1. Making process of tofu and chunggukjang.

Isoflavone extraction

Two grams of pulverized sample were extracted in 10 ml of acetonitrile and 2 ml of 0.1 mol·l^{-1} hydrochloric acid, and stirred at room temperature for 2 h. Each sample was extracted in duplicate. The extract was filtered through a Whatman filter paper (No. 42) (Whatman, Piscataway, New Jersey, USA) and concentrated using a vacuum evaporator. The remaining residue was extracted again with 10 ml of 80% aqueous methanol (HPLC grade; J. T. Baker, Phillipsburg, New Jersey, USA) and filtered through a membrane filter (pore size 0.45 μm; TITAN-Nylon, Gloucester, United Kingdom) [14]. Each filtrate was analysed for isoflavones by HPLC. Although ROSTAGNO et al. [24] reported optimal sample preparation and extraction conditions for isoflavones in various soya matrices, the extraction efficiency of the acidification-based method was comparable for sample types analysed in this study.

HPLC analysis of isoflavones

An HPLC system Acme9000 Vitamin Analyzer (Younglin Instruments, Anyang-si, Korea) with an YMC-Pack ODS AM-303 column (5 μm, 250 mm × 4.6 mm I.D.), equipped with a UV-vis detector set at 254 nm was used for separation and detection of isoflavones. Twenty microliters

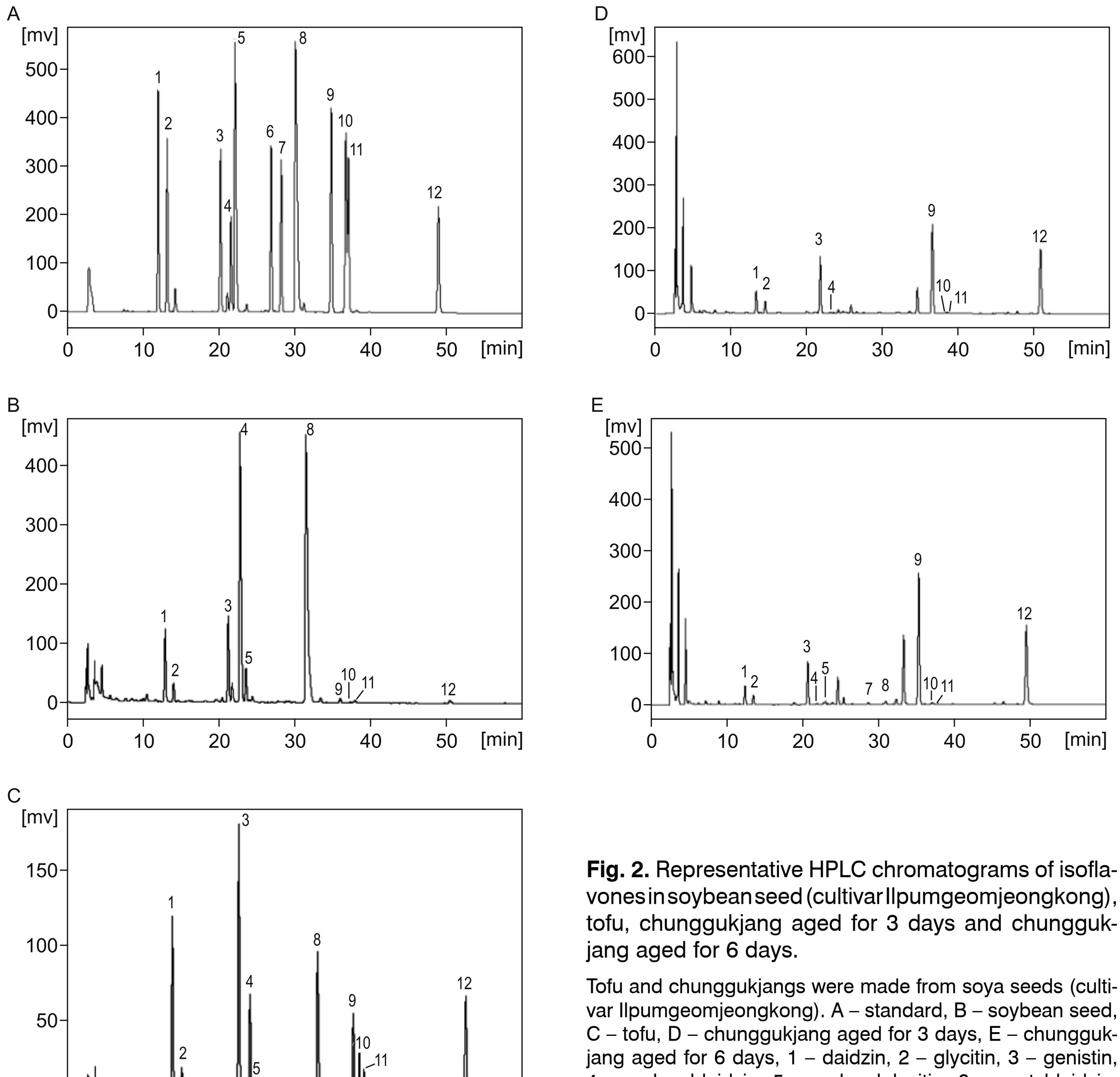

Fig. 2. Representative HPLC chromatograms of isoflavones in soybean seed (cultivar Ilpumgeomjeongkong), tofu, chunggukjang aged for 3 days and chunggukjang aged for 6 days.

Tofu and chunggukjangs were made from soya seeds (cultivar Ilpumgeomjeongkong). A – standard, B – soybean seed, C – tofu, D – chunggukjang aged for 3 days, E – chunggukjang aged for 6 days, 1 – daidzin, 2 – glycitin, 3 – genistin, 4 – malonyldaidzin, 5 – malonylglycitin, 6 – acetyldaidzin, 7 – acetylglycitin, 8 – malonylgenistin, 9 – daidzein, 10 – glycitein, 11 – acetylgenistin, 12 – genistein.

of sample extract were injected, and a stepwise gradient system consisting of 0.1% glacial acetic acid in distilled water (solvent A) and 0.1% glacial acetic acids in acetonitrile (solvent B) were used. The gradient was programmed as follows: start: 85% B; start to 50 min: 35% B; 50–60 min: 35% B. Total HPLC run time was 60 min at a flow rate of 1 ml·min^{-1} [14]. Each sample extract was analysed in duplicate and all solvents were of HPLC grade (J. T. Baker). The representative HPLC chromatograms of 12 isoflavone standards, soya seed, tofu, chunggukjang aged for 3 days and chunggukjang aged for 6 days are shown in Fig. 2.

Quantification of isoflavones

Twelve isoflavone standards were purchased from LC Laboratories (Woburn, Massachusetts, USA) and dissolved in dimethyl sulfoxide. Standards for the construction calibration curves were prepared by serial dilution of each isoflavone standard. All calibration curves had a high linearity ($r^2 \geq 0.998$). Comparison of retention times to the standards was used to identify isoflavones and peak areas were used to quantify them. Total isoflavone contents were expressed as millimoles per kilogram of dry sample (mmol·kg^{-1}, dry basis). The recovery rates of isoflavones in percent were calculated as the contents (mmol·kg^{-1}, dry basis) in final products (tofu, chunggukjang aged for 3 days, chunggukjang aged for 6 days) divided by their contents in soya seeds (mmol·kg^{-1}, dry basis) and multiplied by 100 [25].

Statistical analysis

Statistical analysis was performed using the SAS software package, Version 9.1 (SAS, Cary, North Carolina, USA). Least significant differences (*LSD*) were set at 5% for total and individual isoflavone contents in soya seeds, tofu and chunggukjang aged for 3 or 6 days.

RESULTS

Isoflavone composition of seeds of 20 soya cultivars

The contents and profiles of isoflavones in seeds of 20 soya cultivars are shown in Tab. 1. Soya seeds contained 12.03 mmol·kg^{-1} (equals to 3290 mg·kg^{-1}, aglucon equivalent) of total isoflavones. Total genistein made up 81% (9.73 mmol·kg^{-1}) of total isoflavones, followed by 13.7% (1.65 mmol·kg^{-1}) of total daidzein and 5.3% (0.64 mmol·kg^{-1}) of total glycitein. Since malonylgenistin, a constituent of total genistein, contributed by an impressive 8.14 mmol·kg^{-1} or 68% to the total isoflavones, total genistein was the major constituent of total isoflavones. About 80.3% (9.66 mmol·kg^{-1}) of isoflavones in soya seeds were in the malonylglucoside form, and 14.5% (1.75 mmol·kg^{-1}) were in the glucoside forms. These two were the major isoflavone forms, while aglucon and acetylglucoside were the minor forms representing only 3.9% (0.47 mmol·kg^{-1}) and 1.3% (0.16 mmol·kg^{-1}) of total isoflavones, respectively. The mole percent distribution of isoflavone forms matched the findings of a previous report [25].

Change of isoflavone composition during making of tofu

The isoflavone contents of tofu made with each cultivar are presented in Tab. 2. Whey produced during tofu making was not included in the analyses of total and individual isoflavones. The recovery rate of total isoflavones was about 50% due to the filtration and coagulation steps during tofu making. Tofu contained 5.94 mmol·kg^{-1} (equals to 1595 mg·kg^{-1}, aglucon equivalent) of total isoflavones. Similar to soya seeds, total genistein was also dominant in tofu (3.78 mmol·kg^{-1}), making up 64% of total isoflavones, followed by total daidzein (1.99 mmol·kg^{-1}, 34%) and total glycitein (0.16 mmol·kg^{-1}, 2.7%). Aglucons, the dominant isoflavone forms in tofu, represented 45% of total isoflavones. Glucoside, malonylglucoside and acetylglucoside forms represented 27%, 25% and 3% of total isoflavones found in tofu, respectively. Acetylglucosides (0.19 mmol·kg^{-1}) were the minor components of isoflavones in tofu. In general, β-glucosides, acetyl-β-glucosides and malonyl-β-glucosides were converted to aglucons by heat, fermentation and endogenous enzyme reactions [13, 14, 26, 27]. During the tofu-making process, malonyl-β-glucosides decreased from 9.66 mmol·kg^{-1} in soya seeds to 1.47 mmol·kg^{-1} in tofu, while aglucons increased from 0.47 mmol·kg^{-1} in soya seeds to 2.69 mmol·kg^{-1} in tofu (Tab. 1, 2), indicating that large amounts of malonyl-β-glucosides, in particular malonylgenitin, were lost during tofu making (i. e. filtering, coagulation and whey exclusion).

Change of isoflavone composition during making of chunggukjang

Tab. 3 shows the total and individual isoflavones in 20 chunggukjang samples that were aged for 3 days. On average, chunggukjang aged for 3 days contained 12.38 mmol·kg^{-1} (equals to 3284 mg·kg^{-1}, aglucon equivalent) of total isoflavones. Treatments of fermentation and aging did not affect the total isoflavones in chunggukjang. The recovery rate of total isoflavones

Tab. 1. Contents and profiles of total and individual isoflavones in soya seeds (mmol·kg^{-1}, dry basis).

Cultivar	Classification	Aglucon				Glucoside				Acetylglucoside				Malonylglucoside				Total			
		Dein	Gein	Glein	Total	Din	Gin	Glin	Total	AD	AG	AGl	Total	MD	MG	MGl	Total	TD	TG	TGl	TI
Geumkangkong	P/C	0.49	0.14	0.03	0.65	0.29	2.38	0.10	2.77	0.09	0.02	0.02	0.13	1.57	10.86	0.39	12.81	2.44	13.40	0.53	16.37
Jangsukong	P/C	0.50	0.16	0.04	0.70	0.24	1.52	0.10	1.86	0.06	0.01	Tr	0.07	1.22	8.00	0.33	9.55	2.02	9.68	0.47	12.17
Hwangkeumkong	P/C	0.26	0.13	0.02	0.42	0.12	0.99	0.08	1.19	0.13	0.02	0.01	0.16	0.75	6.74	0.35	7.84	1.26	7.89	0.47	9.61
Baekunkong	P/C	0.32	0.10	0.03	0.46	0.21	1.33	0.08	1.62	ND	0.01	0.01	0.03	1.18	8.29	0.41	9.89	1.71	9.74	0.54	11.99
Jangwonkong	P/C	0.48	0.17	0.04	0.69	0.20	1.49	0.14	1.83	0.07	0.03	0.01	0.10	0.91	8.01	0.37	9.29	1.65	9.70	0.56	11.91
Hojangkong	P/C	0.34	0.10	0.03	0.47	0.21	1.22	0.12	1.54	0.15	Tr	0.01	0.16	1.07	7.55	0.53	9.14	1.76	8.87	0.69	11.32
Mallikong	P/C	1.02	0.26	0.04	1.33	0.36	2.55	0.34	3.25	0.21	0.05	0.11	0.37	2.08	14.65	1.25	17.97	3.67	17.51	1.74	22.92
Jinpumkong 2	P/C	0.39	0.13	0.02	0.54	0.24	1.32	0.19	1.75	0.10	0.04	Tr	0.14	1.37	8.36	0.78	10.50	2.10	9.85	1.00	12.95
Samnamkong	P/C	0.56	0.15	0.03	0.74	0.30	1.53	0.24	2.06	0.07	0.05	0.02	0.14	1.41	9.09	0.77	11.28	2.33	10.82	1.06	14.21
Daemankong	P/C	0.32	0.12	0.02	0.46	0.20	1.58	0.05	1.83	0.06	0.03	0.01	0.10	0.93	7.88	0.22	9.03	1.52	9.61	0.30	11.43
Geomjeongolkong	C/R	0.15	0.03	0.03	0.20	0.03	0.64	0.24	0.91	ND	0.04	Tr	0.04	0.53	3.79	0.37	4.68	0.70	4.50	0.64	5.84
Ilpumgeomjeongkong	C/R	0.26	0.08	0.03	0.37	0.28	1.70	0.15	2.12	0.08	0.07	Tr	0.15	1.26	9.45	0.44	11.15	1.88	11.29	0.63	13.80
Seonheukkong	C/R	0.09	0.06	0.03	0.19	0.08	1.22	0.07	1.37	0.05	0.08	ND	0.13	0.49	7.64	0.40	8.52	0.71	8.99	0.50	10.21
Daechoobamkong	C/R	0.31	0.09	0.04	0.44	0.31	1.61	0.06	1.98	0.02	0.99	ND	1.01	1.37	9.04	0.20	10.62	2.01	11.73	0.30	14.04
Heukseolitaekong	C/R	0.12	0.04	0.03	0.19	0.15	0.86	0.12	1.13	0.04	ND	ND	0.04	0.81	5.82	0.42	7.04	1.12	6.71	0.57	8.41
Chengseolitaekong	C/R	0.19	0.07	0.03	0.29	0.20	1.40	0.10	1.70	ND	0.04	ND	0.04	1.04	9.19	0.35	10.58	1.43	10.70	0.47	12.61
Jinyulkong	C/R	0.11	0.06	0.06	0.23	0.08	0.88	0.18	1.14	0.10	0.05	0.01	0.16	0.57	6.07	0.57	7.22	0.87	7.06	0.82	8.75
Galmikong	C/R	0.10	0.09	0.03	0.22	0.12	0.86	0.09	1.07	0.04	0.01	ND	0.06	0.61	5.36	0.29	6.26	0.88	6.32	0.40	7.60
Cheongdukong 1	C/R	0.21	0.08	0.04	0.33	0.24	1.78	0.18	2.21	0.02	ND	0.01	0.03	1.10	9.36	0.57	11.03	1.57	11.22	0.81	13.60
Cheongjakong 16	C/R	0.28	0.11	0.03	0.42	0.19	1.34	0.09	1.62	0.07	0.05	Tr	0.12	0.89	7.60	0.25	8.74	1.43	9.10	0.37	10.90
Mean		0.33	0.11	0.03	0.47	0.20	1.41	0.14	1.75	0.07	0.08	0.01	0.16	1.06	8.14	0.46	9.66	1.65	9.73	0.64	12.03
Standard deviation		0.22	0.05	0.01	0.27	0.09	0.48	0.07	0.58	0.05	0.21	0.02	0.21	0.40	2.24	0.24	2.75	0.70	2.76	0.33	3.79
Least significant differences, 0.05		0.12	0.01	0.01	0.12	0.01	0.13	0.01	0.13	0.10	0.01	0.02	0.12	0.08	0.25	0.06	0.26	0.19	0.27	0.07	0.34

P/C – soya cultivar group for “paste/curd”; C/R – soya cultivar group for “cooked with rice”.
Abbreviations: Dein – daidzein; Gein – genistein; Glein – glycitein; Din – daidzin; Gin – genistin; Glin – glycitin; AD – acetyldaidzin; AG – acetylgenistin; AGl – acetylglycitin; MD – malonyldaidzin; MG – malonylgenistin; MGl – malonylglycitin; TD – total daidzein (Dein + Din + AD + MD); TG – total genistein (Gein + Gin + AG + MG); TGl – total glycitein (Glein + Glin + AGl + MGl); TI – total isoflavones (TD + TG + TGl); ND – not detected, Tr – trace amounts.

Tab. 2. Contents and profiles of total and individual isoflavones of 20 kinds of tofu (mmol·kg^{-1}, dry basis).

Cultivar	Classification	Aglucon				Glucoside				Acetylglucoside				Malonylglucoside				Total			
		Dein	Gein	Glein	Total	Din	Gin	Glin	Total	AD	AG	AGl	Total	MD	MG	MGl	Total	TD	TG	TGl	TI
Geumkangkong	P/C	2.23	1.35	0.04	3.62	0.14	1.22	0.03	1.38	0.08	0.06	ND	0.14	0.20	1.77	0.05	2.03	2.66	4.39	0.13	7.17
Jangsukong	P/C	1.82	0.93	0.05	2.80	0.19	1.53	0.04	1.76	0.16	0.07	ND	0.23	0.14	1.09	0.03	1.27	2.31	3.62	0.13	6.06
Hwangkeumkong	P/C	1.22	1.00	0.04	2.26	0.10	1.37	0.03	1.51	0.07	0.06	ND	0.12	0.12	1.43	0.04	1.59	1.50	3.86	0.12	5.48
Baekunkong	P/C	1.32	0.82	0.04	2.18	0.14	1.19	0.03	1.36	0.09	0.05	ND	0.14	0.15	1.22	0.05	1.42	1.70	3.28	0.13	5.10
Jangwonkong	P/C	1.77	1.18	0.05	3.00	0.15	1.39	0.05	1.59	0.10	0.08	ND	0.17	0.12	1.19	0.05	1.37	2.14	3.84	0.15	6.13
Hojangkong	P/C	1.86	1.13	0.05	3.04	0.16	1.46	0.06	1.67	0.13	0.08	ND	0.21	0.13	1.07	0.06	1.26	2.27	3.74	0.17	6.18
Mallikong	P/C	2.57	1.63	0.07	4.27	0.19	1.71	0.09	1.99	0.13	0.13	ND	0.26	0.23	1.93	0.13	2.28	3.13	5.39	0.29	8.80
Jinpumkong 2	P/C	1.10	0.54	0.05	1.70	0.16	1.27	0.08	1.51	0.12	0.08	ND	0.19	0.10	0.70	0.06	0.86	1.48	2.58	0.19	4.26
Samnamkong	P/C	2.13	1.03	0.06	3.23	0.21	1.80	0.09	2.10	0.16	0.11	ND	0.27	0.16	1.26	0.08	1.50	2.67	4.19	0.24	7.09
Daemankong	P/C	1.69	1.18	0.05	2.91	0.16	1.82	0.03	2.01	0.14	0.07	ND	0.21	0.10	0.97	0.03	1.09	2.08	4.05	0.10	6.22
Geomjeongolkong	C/R	1.17	0.64	0.06	1.87	0.06	0.68	0.05	0.79	0.04	0.08	ND	0.12	0.13	1.42	0.08	1.64	1.40	2.83	0.19	4.42
Ilpumgeomjeongkong	C/R	1.88	1.06	0.07	3.01	0.26	2.16	0.09	2.50	0.20	0.12	ND	0.31	0.17	1.68	0.06	1.91	2.51	5.02	0.22	7.74
Seonheukkong	C/R	0.65	0.93	0.05	1.63	0.08	2.15	0.04	2.27	0.06	0.07	ND	0.12	0.07	1.37	0.04	1.48	0.85	4.52	0.14	5.51
Daechoobamkong	C/R	2.95	1.44	0.06	4.46	0.16	1.30	0.02	1.48	0.11	0.11	ND	0.22	0.19	1.56	0.03	1.78	3.40	4.41	0.12	7.94
Heukseolitaekong	C/R	0.99	0.54	0.05	1.59	0.09	0.85	0.06	1.00	0.09	0.07	ND	0.16	0.09	0.77	0.06	0.92	1.27	2.23	0.17	3.66
Chengseolitaekong	C/R	1.31	0.85	0.04	2.21	0.13	1.39	0.03	1.56	0.09	0.03	ND	0.13	0.15	1.81	0.04	2.01	1.69	4.09	0.12	5.90
Jinyulkong	C/R	1.27	1.06	0.06	2.39	0.05	0.88	0.05	0.98	0.06	0.11	Tr	0.18	0.06	0.88	0.06	1.00	1.43	2.93	0.18	4.54
Galmikong	C/R	1.06	0.86	0.06	1.99	0.06	0.90	0.03	0.99	0.05	0.11	Tr	0.17	0.08	1.07	0.04	1.19	1.26	2.94	0.13	4.34
Cheongdukong 1	C/R	1.92	1.32	0.06	3.30	0.14	1.67	0.07	1.88	0.18	0.10	Tr	0.28	0.12	1.34	0.06	1.51	2.36	4.42	0.19	6.97
Cheongjakong 16	C/R	1.41	0.89	0.04	2.35	0.12	1.32	0.03	1.47	0.11	0.06	ND	0.16	0.11	1.07	0.03	1.20	1.74	3.34	0.10	5.19
Mean		1.62	1.02	0.05	2.69	0.14	1.40	0.05	1.59	0.11	0.08	–	0.19	0.13	1.28	0.05	1.47	1.99	3.78	0.16	5.94
Standard deviation		0.57	0.28	0.01	0.82	0.05	0.41	0.02	0.45	0.04	0.03	–	0.06	0.04	0.35	0.02	0.39	0.67	0.82	0.05	1.54
Least significant differences, 0.05		0.03	0.02	0.01	0.04	0.02	0.22	0.00	0.22	0.03	0.02	–	0.04	0.01	0.15	0.01	0.15	0.05	0.27	0.01	0.27

P/C – soya cultivar group for “paste/curd”; C/R – soya cultivar group for “cooked with rice”.
Abbreviations: Dein – daidzein; Gein – genistein; Glein – glycitein; Din – daidzin; Gin – genistin; Glin – glycitin; AD – acetyldaidzin; AG – acetylgenistin; AGl – acetylglycitin; MD – malonyldaidzin; MG – malonylgenistin; MGl – malonylglycitin; TD – total daidzein (Dein + Din + AD + MD); TG – total genistein (Gein + Gin + AG + MG); TGl – total glycitein (Glein + Glin + AGl + MGl); TI – total isoflavones (TD + TG + TGl); ND – not detected, Tr – trace amounts.

Tab. 3. Contents and profiles of total and individual isoflavones of 20 kinds of chungkukjang aged for 3 days ($mmol \cdot kg^{-1}$, dry basis).

Cultivar	Classification	Aglucon				Glucoside				Acetylglucoside				Malonylglucoside				Total			
		Dein	Gein	Glein	Total	Din	Gin	Glin	Total	AD	AG	AGl	Total	MD	MG	MGl	Total	TD	TG	TGl	TI
Geumkangkong	P/C	14.55	5.71	0.09	20.35	0.10	1.24	0.12	1.46	ND	0.03	ND	0.03	ND	ND	0.01	0.01	14.64	6.98	0.22	21.84
Jangsukong	P/C	10.58	3.84	0.08	14.50	0.22	1.98	0.12	2.33	ND	0.01	ND	0.01	0.03	ND	ND	0.03	10.83	5.84	0.20	16.88
Hwangkeumkong	P/C	4.15	2.90	0.06	7.11	0.17	2.17	0.10	2.44	ND	ND	ND	ND	0.07	ND	0.01	0.08	4.39	5.07	0.17	9.64
Baekunkong	P/C	7.28	3.03	0.06	10.37	0.16	1.51	0.13	1.79	ND	0.02	ND	0.02	0.04	ND	0.01	0.04	7.48	4.55	0.20	12.23
Jangwonkong	P/C	8.98	3.75	0.10	12.83	0.13	1.93	0.14	2.19	0.01	0.02	ND	0.03	ND	ND	0.01	0.01	9.13	5.70	0.25	15.07
Hojangkong	P/C	9.81	4.07	0.09	13.97	0.05	0.82	0.13	1.00	0.01	ND	ND	0.01	ND	ND	0.01	0.01	9.87	4.90	0.22	14.99
Mallikong	P/C	18.73	7.68	0.17	26.58	0.08	1.18	0.30	1.56	0.02	0.02	ND	0.04	0.01	ND	0.01	0.02	18.84	8.88	0.48	28.19
Jinpumkong 2	P/C	7.47	2.55	0.11	10.13	0.32	2.31	0.35	2.98	0.02	ND	ND	0.02	0.07	ND	0.01	0.08	7.87	4.86	0.47	13.20
Samnamkong	P/C	5.47	2.14	0.08	7.68	0.32	2.24	0.29	2.85	ND	ND	ND	ND	0.14	ND	0.01	0.15	5.93	4.38	0.37	10.67
Daemankong	P/C	9.05	4.42	0.06	13.52	0.07	1.45	0.07	1.59	ND	ND	ND	ND	ND	ND	0.01	0.01	9.12	5.87	0.14	15.13
Geomjeongolkong	C/R	2.22	0.82	0.03	3.07	0.06	1.09	0.09	1.23	ND	0.02	ND	0.02	ND	0.03	ND	0.03	2.27	1.95	0.12	4.35
Ilpumgeomjeongkong	C/R	7.13	2.45	0.04	9.62	0.10	1.51	0.13	1.74	0.02	ND	ND	0.02	ND	0.04	ND	0.04	7.25	4.00	0.17	11.42
Seonheukkong	C/R	2.64	1.96	0.03	4.64	0.07	2.04	0.10	2.21	ND	ND	ND	ND	ND	0.04	0.01	0.05	2.71	4.04	0.15	6.90
Daechoobamkong	C/R	9.19	3.09	0.06	12.34	0.09	1.21	0.05	1.35	0.02	ND	ND	0.02	ND	0.15	ND	0.15	9.29	4.45	0.12	13.86
Heukseolitaekong	C/R	4.23	1.21	0.04	5.48	0.06	0.97	0.12	1.14	ND	0.03	ND	0.03	ND	0.02	ND	0.02	4.29	2.22	0.16	6.67
Chengseolitaekong	C/R	7.35	3.05	0.05	10.45	0.07	1.29	0.07	1.42	ND	0.04	ND	0.04	ND	0.01	0.02	0.04	7.42	4.40	0.14	11.96
Jinyulkong	C/R	2.80	1.46	0.07	4.33	0.02	0.77	0.12	0.91	ND	ND	ND	ND	ND	0.02	ND	0.02	2.82	2.25	0.18	5.26
Galmikong	C/R	4.04	1.82	0.10	5.96	0.04	1.04	0.06	1.14	ND	ND	ND	ND	ND	0.34	0.03	0.37	4.08	3.20	0.19	7.47
Cheongdukong 1	C/R	8.69	3.43	0.08	12.20	0.05	0.96	0.13	1.14	ND	0.01	ND	0.01	ND	0.03	0.05	0.08	8.73	4.42	0.27	13.42
Cheongjakong 16	C/R	4.17	1.90	0.03	6.10	0.14	2.02	0.09	2.25	ND	0.03	ND	0.03	ND	0.06	0.01	0.08	4.30	4.01	0.14	8.46
Mean		7.43	3.06	0.07	10.56	0.12	1.49	0.14	1.74	0.02	0.02	–	0.02	0.02	0.04	0.01	0.07	7.56	4.60	0.22	12.38
Standard deviation		4.12	1.61	0.03	5.70	0.09	0.51	0.08	0.62	0.01	0.01	–	0.01	0.04	0.08	0.01	0.08	4.12	1.62	0.11	5.85
Least significant differences, 0.05		0.06	0.03	0.01	0.07	0.01	0.02	0.01	0.02	0.01	0.01	–	0.01	0.02	0.01	0.01	0.02	0.06	0.04	0.01	0.08

P/C – soya cultivar group for "paste/curd"; C/R – soya cultivar group for "cooked with rice".
Abbreviations: Dein – daidzein; Gein – genistein; Glein – glycitein; Din – daidzin; Gin – genistin; Glin – glycitin; AD – acetyldaidzin; AG – acetylgenistin; AGl – acetylglycitin; MD – malonyldaidzin; MG – malonylgenistin; MGl – malonylglycitin; TD – total daidzein (Dein + Din + AD + MD); TG – total genistein (Gein + Gin + AG + MG); TGl – total glycitein (Glein + Glin + AGl + MGl); TI – total isoflavones (TD + TG + TGl); ND – not detected, Tr – trace amounts.

Tab. 4. Contents and profiles of total and individual isoflavones of 20 kinds of chungkukjang aged for 6 days ($mmol \cdot kg^{-1}$, dry basis).

Cultivar	Classification	Aglucon				Glucoside				Acetylglucoside				Malonylglucoside				Total			
		Dein	Gein	Glein	Total	Din	Gin	Glin	Total	AD	AG	AGl	Total	MD	MG	MGl	Total	TD	TG	TGl	TI
Geumkangkong	P/C	14.90	5.11	0.09	20.10	0.12	1.33	0.11	1.57	0.02	0.02	0.01	0.05	ND	ND	ND	ND	15.04	6.47	0.21	21.72
Jangsukong	P/C	10.80	3.54	0.07	14.42	0.22	1.84	0.12	2.17	0.02	0.06	0.02	0.10	ND	ND	ND	ND	11.04	5.44	0.21	16.69
Hwangkeumkong	P/C	4.41	3.00	0.06	7.47	0.16	1.93	0.09	2.17	0.01	0.04	0.01	0.06	ND	ND	ND	ND	4.58	4.97	0.16	9.70
Baekunkong	P/C	10.39	4.18	0.07	14.63	0.14	1.29	0.12	1.56	0.02	0.07	0.03	0.12	ND	ND	ND	ND	10.55	5.54	0.21	16.31
Jangwonkong	P/C	8.84	3.35	0.09	12.29	0.13	1.95	0.13	2.22	0.01	0.06	0.01	0.08	ND	ND	ND	ND	8.99	5.37	0.23	14.59
Hojangkong	P/C	10.57	4.18	0.10	14.84	0.06	0.87	0.13	1.06	0.02	0.06	0.01	0.09	ND	ND	ND	ND	10.64	5.10	0.24	15.98
Mallikong	P/C	16.65	6.61	0.16	23.42	0.07	1.25	0.24	1.56	0.02	0.01	0.01	0.04	ND	ND	ND	ND	16.74	7.87	0.42	25.03
Jinpumkong 2	P/C	10.17	3.34	0.12	13.63	0.33	2.29	0.45	3.07	0.02	0.08	0.06	0.16	ND	ND	ND	ND	10.52	5.71	0.63	16.86
Samnamkong	P/C	8.95	3.53	0.08	12.55	0.31	2.13	0.28	2.72	0.02	0.02	0.10	0.14	0.03	ND	ND	0.03	9.31	5.68	0.46	15.45
Daemankong	P/C	9.81	4.75	0.06	14.62	0.07	1.33	0.06	1.46	0.02	0.01	0.06	0.09	ND	ND	ND	ND	9.90	6.10	0.18	16.17
Geomjeongolkong	C/R	2.35	0.79	0.04	3.18	0.04	0.72	0.07	0.83	0.02	0.05	0.02	0.10	ND	ND	0.01	0.01	2.42	1.56	0.14	4.12
Ilpumgeomjeongkong	C/R	8.64	2.62	0.05	11.30	0.07	0.97	0.09	1.13	0.02	0.10	0.04	0.16	ND	0.02	0.01	0.03	8.73	3.70	0.19	12.62
Seonheukkong	C/R	2.19	1.53	0.03	3.74	0.05	1.29	0.08	1.42	0.01	0.07	0.05	0.14	ND	0.01	0.01	0.03	2.25	2.90	0.18	5.33
Daechoobamkong	C/R	10.73	2.67	0.06	13.46	0.10	1.06	0.05	1.21	0.02	0.06	0.11	0.19	ND	0.11	ND	0.11	10.84	3.90	0.22	14.96
Heukseolitaekong	C/R	4.65	1.11	0.07	5.83	0.02	0.43	0.07	0.52	0.01	0.06	0.03	0.10	ND	ND	ND	ND	4.69	1.60	0.17	6.45
Chengseolitaekong	C/R	7.11	2.03	0.05	9.20	0.07	1.10	0.06	1.23	0.02	0.06	0.09	0.17	ND	ND	ND	ND	7.20	3.19	0.20	10.59
Jinyulkong	C/R	2.99	1.27	0.06	4.33	0.02	0.62	0.12	0.76	0.02	0.05	0.01	0.08	0.02	ND	ND	0.02	3.06	1.94	0.19	5.20
Galmikong	C/R	3.87	1.33	0.07	5.26	0.03	0.72	0.05	0.80	0.00	0.05	0.05	0.10	ND	0.13	0.02	0.15	3.90	2.23	0.18	6.31
Cheongdukong 1	C/R	8.04	2.32	0.09	10.44	0.07	1.08	0.13	1.28	0.02	0.07	ND	0.09	ND	ND	0.02	0.02	8.12	3.46	0.24	11.82
Cheongjakong 16	C/R	5.25	2.06	0.04	7.34	0.11	1.40	0.08	1.59	0.02	0.06	0.04	0.12	ND	0.02	0.01	0.02	5.38	3.54	0.16	9.08
Mean		8.06	2.97	0.07	11.10	0.11	1.28	0.13	1.52	0.02	0.05	0.04	0.11	Tr	0.01	0.00	0.02	8.19	4.31	0.24	12.75
Standard deviation		3.97	1.51	0.03	5.40	0.09	0.52	0.10	0.66	0.01	0.02	0.03	0.04	–	0.04	0.01	0.04	4.00	1.77	0.12	5.89
Least significant differences, 0.05		0.07	0.04	0.01	0.08	0.01	0.13	0.05	0.14	0.01	0.01	0.02	0.03	–	0.003	0.00	0.02	0.07	0.13	0.05	0.19

P/C – soya cultivar group for “paste/curd”; C/R – soya cultivar group for “cooked with rice”.
Abbreviations: Dein – daidzein; Gein – genistein; Glein – glycitein; Din – daidzin; Gin – genistin; Glin – glycitin; AD – acetyldaidzin; AG – acetylgenistin; AGl – acetylglycitin; MD – malonyldaidzin; MG – malonylgenistin; MGl – malonylglycitin; TD – total daidzein (Dein + Din + AD + MD); TG – total genistein (Gein + Gin + AG + MG); TGl – total glycitein (Glein + Glin + AGl + MGl); TI – total isoflavones (TD + TG + TGl); ND – not detected, Tr – trace amounts.

was about 103%. Individual isoflavone contents and profiles of chunggukjang aged for 3 days differed from those of soya seeds. In chunggukjang aged for 3 days, total isoflavones consisted of total daidzein (7.56 $mmol\cdot kg^{-1}$, 61%), total genistein (4.60 $mmol\cdot kg^{-1}$, 37%) and total glycitein (0.22 $mmol\cdot kg^{-1}$, 1.78%). Daidzein, a constituent of total daidzein, contributed by 60% to total isoflavones in chunggukjang. About 85% of isoflavones were in the aglucon form, 14% in the glucoside form, while acetylglucoside and malonylglucoside forms contributed by less than 1%.

Tab. 4 presents total and individual isoflavone contents of 20 chunggukjang samples aged for 6 days. Chunggukjang aged for 6 days contained 12.75 $mmol\cdot kg^{-1}$ total isoflavones (equals to 3371 $mg\cdot kg^{-1}$, aglucon equivalent). Similar chunggukjang aged for 3 days, there was no effect of fermentation and aging on total isoflavones in chunggukjang aged for 6 days. The recovery rate of total isoflavones was ~106%. In the chunggukjang aged for 6 days, total isoflavones consisted of total daidzein (8.19 $mmol\cdot kg^{-1}$, 64%), total genistein (4.31 $mmol\cdot kg^{-1}$, 34%) and total glycitein (1.9%). A portion of about 87% of isoflavones in this chunggukjang was in the aglucon form and 11.9% in the glucoside form. Malonyl-β-glucoside and acetyl-β-glucoside forms were detected only in trace amounts representing less than 1% of total isoflavones. The contents of four isoflavone groups in chunggukjang were significantly different from each other ($P < 0.05$).

During the process of making chunggukjang, malonyl-β-glucosides decreased from 9.66 $mmol\cdot kg^{-1}$ in soya seeds to 0.07 $mmol\cdot kg^{-1}$ and 0.02 $mmol\cdot kg^{-1}$ in chunggukjang aged for 3 and 6 days, respectively.The decrease in the content of malonyl-β-glucosides was not significant between chunggukjang aged for 3 and 6 days ($P > 0.05$). In contrast to this, aglucons increased significantly from 0.47 $mmol\cdot kg^{-1}$ in soya seeds to 10.56 $mmol\cdot kg^{-1}$ and 11.10 $mmol\cdot kg^{-1}$ in chunggukjang aged for 3 and 6 days, respectively. This indicates that malonyl-β-glucosides were not lost when making chunggukjang. Malonyl-β-glucosides were converted to aglucons during the fermentation and aging process. Interestingly, malonylgenistin decreased significantly from soya seeds to chunggukjang aged for 3 or 6 days, however, the increase in daidzein was greater than the increase in genistein. The reason for this phenomenon is not clear (Tab. 1, 3, and 4). The group effect ("P/C" versus "C/R") on total isoflavones was observed in chunggukjang aged for 3 and 6 days ($P < 0.01$), but not in soya seeds or tofu (data not shown).

DISCUSSION

Processes such as heat treatment, soaking in water, salting, de-foaming, enzymic hydrolysis, fermentation, pressure, etc. are known to affect the content and profiles of isoflavones in soya-based foods [14, 25, 28, 29]. It is also known that heat treatment, such as moist heat, converts more malonyl-β-glucosides to β-glucosides than dry heat methods, which converts only limited amounts of malonyl-β-glucosides to acetyl-β-glucosides [13, 16, 30]. Microbial activity converts malonyl-β-glucosides to aglucons [21, 31, 32]. In addition, the presence of alkali is also known to convert β-glucosides to aglucons [26].

Soaking soya seeds in water prior to heat treatment the tofu-making process converts β-glucosides to aglucons because the endogenous β-glycosidase in soya seeds is activated by water. However, during heat treatment, the change in the amount of β-glucosides is negligible since the loss of β-glucosides is compensated by the formation of β-glucosides from malonyl-β-glucosides [13, 16]. According to HAN et al. [18] and WANG and MURPHY [14], 12% of isoflavones were lost during soaking, 15–20% in the residues of soya curd, 24% during heat treatment (120 °C), 30–31% in whey, 44% through coagulation of tofu and 49% during heating in the production of tempeh. The presence of water during cooking, salting in water, de-foaming or excessive heat treatment also decreased the content of total isoflavones in soya-based foods [13, 33, 34], in particular aglucons, which are lost by leaching in water [13].

In the present study, the increase (%, mole basis) of aglucons and the decrease (%, mole basis) of malonyl-β-glucosides during tofu and chunggukjang making were similar to previous studies [18, 30–33, 35]. In the present study, the contents of total isoflavones (mole basis) in tofu samples were slightly higher than those of whey or the residues of soya curd [18]. In addition, in the present study, total isoflavones (%, mole basis) of chunggukjang increased by ~20% in the cultivars for "P/C making", while decreased by ~17% in those for "C/R". This indicates that some genetic properties of soya cultivars affect the change of total isoflavone content during the making process of soya-based food products.

In the present study, daidzein and genistein contents in chunggukjang aged for 3 days and 6 days were from 3- to 5-fold higher than those of tofu, which is consistent with a previous report [36]. Daidzein contents of chunggukjang aged for 3 days and 6 days were 7.43 $mmol\cdot kg^{-1}$ and 8.06 $mmol\cdot kg^{-1}$, respectively, which were approximately 5-fold higher than the total daidzein content (daidzein (Dein) + daidzin (Din) + malonyldaidzin (MD) + acetyldaidzin (AD) = 1.65 mmol·kg^{-1}) in soya seeds (Tables 1, 3, and 4). On the other hand, contents of genistein in chunggukjang aged for 3 days (3.06 mmol·kg^{-1}) and 6 days (2.97 mmol·kg^{-1}) were approximately 3-fold lower than the total genistein content (genistein (Gein) + genistin (Gin) + malonylgenistin (MG) + acetylgenistin (AG) = 9.73 mmol·kg^{-1}) in soya seeds (Tables 1, 3, and 4). In general, isoflavone profiles were altered by processing between soya seeds to soya-based foods (by heating, fermentation etc.), but the total isoflavone content did not change, if there was no mass loss during the production [14]. In the present study, there was no mass loss during the chunggukjang production, and also the change in total isoflavone contents by processing soya seeds to chunggukjangs was not significant. However, it is not clear why the daidzein content in chunggukjang aged for 3 days and 6 days significantly increased compared to total daidzein in soya seeds.

Although the bioavailability of aglucons and β-glucosides remains controversial, aglucons are effectively absorbed by humans compared to β-glucosides [37–39]. Gu et al. [40] reported that genistein and daidzein possess higher estrogen receptor-binding affinity than the β-glucoside form. In addition, according to Xu et al. [41], daidzein is better bioavailable in adult women than genistein. Hence, chunggukjang provides more nourishment than soya seeds or tofu, because it contains large amounts of aglucons like daidzein and genistein.

In conclusion, the present study extended the knowledge on the changes of total and individual isoflavone contents and their profiles in soya seeds, tofu and chunggukjang depending on the treatment of processing, fermentation or aging. Fermentation and aging period did not affect the total isoflavone contents in chunggukjang, whereas processing like filtering and coagulation during tofu making decreased the total isoflavone content by ~50%. Isoflavone profiles, in particular contents of daidzein and genistein, were different in soya seeds, tofu, chunggukjang aged for 3 and chunggukjang aged for 6 days. Interestingly, daidzein contents in chunggukjang aged for 3 and 6 days were approximately 5-fold higher than the total daidzein content (Dein + Din + MD + AD) in soya seeds. By contrast, genistein contents in chunggukjang aged for 3 and 6 days were approximately 3-fold lower than the total genistein content (Gein + Gin + MG + AG) in soya seeds due to an unknown mechanism. Consequently, chunggukjang has a higher nutritive value than soya seeds or tofu, because it contains the better bioavailable isoflavone aglucons such as daidzein or genistein.

Acknowledgments

This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (No. 2010-0010122).

REFERENCES

1. Wu, A. H. – Yu, M. C. – Tseng, C. C. – Pike, M. C.: Epidemiology of soy exposures and breast cancer risk. British Journal of Cancer, *98*, 2008, pp. 9–14.
2. Adlercreutz, H. – Mazur, W.: Phyto-oestrogens and Western diseases. Annals of Medicine *29*, 1997, pp. 95–120.
3. Wildman, R. E. C.: Handbook of nutraceuticals and functional foods. In: Hendrich, S. – Murphy, P. A.: Isoflavones. Boca Raton: CRC Press, 2007, pp. 23–54.
4. Arjmandi, B. H. – Alekel, L. – Hollis, B. W. – Amin, D. – Stacewicz-Sapuntzakis, M. – Guo, P. – Kukreja, S. C.: Dietary soybean protein prevents bone loss in an ovariectomized rat model of osteoporosis. Journal of Nutrition, *126*, 1996, pp. 161–167.
5. Messina, M.: Soyfoods, soybean isoflavones, and bone health. Korean Soybean Digest, *15*, 1998, pp. 122–136.
6. Liu, K.: Soybeans as functional foods and ingredients. In: Liu, K.: Soy isoflavones: Chemistry, processing effects, health benefits, and commercial production. Champaign: AOCS Press, 2004, pp. 52–72.
7. Choi, J. S. – Kwon, T. W. – Kim, J. S.: Isoflavone contents in some cultivars of soybean. Food Science and Biotechnology, *5*, 1996, pp. 167–169.
8. Setchell, K. D. R.: Phytoestrogens in the prevention of hormone dependent disease with reference to the significance to soy infant formula diets. Korean Soybean Digest, *15*, 1998, pp. 137–146.
9. Lee, S. J. – Ahn, J. K. – Kim, S. H. – Kim, J. T. – Han, S. J. – Jung, M. Y. – Chung, I. M.: Variation in isoflavone of soybean cultivars with location and storage duration. Journal of Agricultural and Food Chemistry, *51*, 2003, pp. 3382–3389.
10. Wang, H. J. – Murphy, P. A.: Isoflavone composition of American and Japanese soybeans in Iowa: Effects of variety, crop year, and location. Journal of Agricultural and Food Chemistry, *42*, 1994, pp. 1674–1677.
11. Tsukamoto, C. – Shimada, S. – Igita, K. – Kudou, S. – Kokubun, M. – Okubo, K. – Kitamura, K.: Factors affecting isoflavone content in soybean seeds: Changes in isoflavones, saponins, and composition of fatty acids at different temperatures during seed development. Journal of Agricultural and Food Chemistry, *43*, 1995, pp. 1184–1192.
12. Xu, Z. – Wu, Q. – Godber, J. S.: Stabilities of

daidzin, glycitin, genistin, and generation of derivatives during heating. Journal of Agricultural and Food Chemistry, *50*, 2002, pp. 7402–7406.
13. Grun, I. G. – Adhikari, K. – Li, C. – Li, Y. – Lin, B. – Zhang, J. – Fernando, L. N.: Changes in the profile of genistein, daidzein, and their conjugates during thermal processing of tofu. Journal of Agricultural and Food Chemistry, *49*, 2001, pp. 2839–2843.
14. Wang, H. J. – Murphy, P. A.: Mass balance study of isoflavones during soybean processing. Journal of Agricultural and Food Chemistry, *44*, 1996, pp. 2377–2383.
15. Wang, H. J. – Murphy, P. A.: Isoflavone contents in commercial soybean foods. Journal of Agricultural and Food Chemistry, *42*, 1994, pp. 1666–1673.
16. Coward, L. – Barnes, N. C. – Setchell, K. D. R. – Barnes, S.: Genistein, daidzein, and their β–glycoside conjugates: Antitumor isoflavones in soybean foods from American and Asian diets. Journal of Agricultural and Food Chemistry, *41*, 1993, pp. 1961–1967.
17. USDA-Iowa State University Database on the isoflavone content of foods. Release 1.3.2002 [online]. Beltsville, Maryland : U.S. Department of Agriculture, Agricultural Research Service, 2002 [cit. April 05, 2009]. <http://www.nal.usda.gov/fnic/foodcomp/Data/isoflav/isoflav.html>
18. Han, J. S. – Hong, H. D. – Kim, S. R.: Changes in isoflavone contents and mass balance during soybean processing. Food Science and Biotechnology, *16*, 2007, pp. 426–433.
19. Franke, A. A. – Hankin, J. H. – Yu, M. C. – Maskarinec, G. – Low, S. H. – Custer, L. J.: Isoflavone levels in soy foods consumed by multiethnic populations in Singapore and Hawaii. Journal of Agricultural and Food Chemistry, *47*, 1999, pp. 977–986.
20. Kim, J. S. – Yoo, S. M. – Choe, J. S. – Park, H. J. – Hong, S. P. – Chang, C. M.: Physicochemical properties of traditional chongugjang produced in different regions. Agricultural and Chemical Biotechnology, *41*, 1998, pp. 377–383.
21. Kim, S. H. – Yang, J. L. – Song, Y. S.: Physiological functions of cheonggukjang. Food Industry and Nutrition, *4*, 1999, pp. 40–46.
22. Chung, I. M. – Seo, S. H. – Ahn, J. K. – Kim, S. H.: Effect of processing, fermentation, and aging treatment to content and profile of phenolic compounds in soybean seed, soy curd and soy paste. Food Chemistry, *127*, 2011, pp. 960–967.
23. Lee, S. J. – Philippe, S. – Kim, J. J. – Moon, H. I. – Roe, H. M. – Kim, E. H. – Seo, S. H. – Kang, E. Y. – Ahn, J. K. – Chung, I. M.: Isoflavones in Korean soybeans differing in seed coat and cotyledon color. Journal of Food Composition and Analysis, *23*, 2009, pp. 160–165.
24. Rostagno, M. A. – Villares, A. – Guillamon, E. – Garcia-Lafuente, A. – Martinez, J. A.: Sample preparation for the analysis of isoflavones from soybeans and soy foods. Journal of Chromatography A, *1216*, 2009, pp. 2–29.
25. Jung, S. – Murphy, P. A. – Sala, I.: Isoflavone profiles of soymilk as affected by high-pressure treatments of soymilk and soybeans. Food Chemistry, *111*, 2008, pp. 592–598.
26. Yang, S. Q. – Wang, L. J. – Yan, Q. J. – Jiang, Z. Q. – Li, L.: Hydrolysis of soybean isoflavone glycosides by a thermalstable β-glucosidase from *Paecilomyces thermophila*. Food Chemistry, *115*, 2009, pp. 1247–1252.
27. Yin, L.-J. – Li, L.-T. – Liu, H. – Saito, M. – Tatsumi, E.: Effects of fermentation temperature on the content and composition of isoflavones and β-glucosidase activity in sufu. Bioscience, Biotechnology and Biochemistry, *69*, 2005, pp. 267–272.
28. Shimoni, E.: Stability and shelf life of bioactive compounds during food processing and storage: Soy isoflavones. Journal of Food Science, *69*, 2004, pp. R160–R165.
29. Barnes, S. – Kirk, M. – Coward, L.: Isoflavones and their conjugation soy foods: extraction conditions and analysis by HPLC-mass spectrometry. Journal of Agricultural and Food Chemistry, *42*, 1994, pp. 2466–2474.
30. Mathias, K. – Ismail, B. – Corvalan, C. M. – Hayes, K. D.: Heat and pH effects on the conjugated forms of genistin and daidzin isoflavones. Journal of Agricultural and Food Chemistry, *54*, 2006, pp. 7495–7502.
31. Yang, S. O. – Lee, S. W. – Park, Y. W. – Lee, S. J. – Chang, P. S. – Choi, S. S.: Succinyl daidzin and succinyl genistin are new isoflavone derivatives found in chunggukjang. Food Science and Biotechnology, *17*, 2008, pp. 172–175.
32. Jang, C. H. – Lim, J. K. – Kim, J. H. – Park, C. S. – Kwon, D. Y. – Kim, Y. S.: Change of isoflavone content during manufacturing of chunggukjang, a traditional fermented soy food. Food Science and Biotechnology, *15*, 2006, pp. 643–646.
33. Yang, S. O. – Chang, P. S. – Lee, J. H.: Isoflavone distribution and β-glucosidase activity in cheonggukjang, a traditional Korean whole soybean-fermented food. Food Science and Biotechnology, *15*, 2006, pp. 96–101.
34. Coward, L. – Smith, M. – Kirk, M. – Barnes, S.: Chemical modification of isoflavones in soyfoods during cooking and processing. American Journal of Clinical Nutrtion, *15*, 1988, pp. 1486s–1491s.
35. Kim, J. S. – Yoon, S.: Isoflavone contents and β-glucosidase activities in soybean, meju and doenjang. Korean Journal of Food Science and Technology, *31*, 1999, pp. 1405–1409.
36. Choi, Y. B. – Sohn, H. S.: Isoflavone content in Korean fermented and unfermented soybean foods. Korean Journal of Food Science and Technology, *30*, 1998, pp. 745–750.
37. Nielsen, I. L. – Williamson, G.: Review of the factors affecting bioavailability if soy isoflavones in humans. Nutrition and Cancer-An International Journal, *57*, 2007, pp. 1–10.
38. Kawakami, Y. – Tsurugasaki, W. – Nakamura, S. – Osada, K.: Comparison of regulative functions between dietary soy isoflavones aglycone and glucoside in lipid metabolism in rats fed cholesterol. Journal of Nutrional Biochemistry, *16*, 2005, pp. 205–212.

39. Izumi, T. – Piskula, M. K. – Osawa, S. – Obata, A. – Tobe, K. – Saito, M. – Kataoka, S. – Kubota, Y. – Kikuchi, M.: Soy isoflavone aglycones are absorbed faster and higher amounts than their glucosides in humans. Journal of Nutrition, *130*, 2000, pp. 1695–1699.
40. Gu, L. – House, S. E. – Prior, R. L. – Fang, N. – Ronis, M. J. J. – Clarkson, T. B. – Wilson, M. E. – Badger, T. M.: Metabolic phenotype of isoflavones differ among female rats, pigs, monkeys, and women. Journal of Nutrtion, *136*, 2006, pp. 1215–1221.
41. Xu, X. – Wang, H. – Murphy, P. A. – Cook, L. – Hendrich, S.: Daidzein is a more bioavailable soymilk isoflavone than is genistein in adult women. Journal of Nutrtion, *124*, 1994, pp. 825–832.

Received 12 November 2011; revised 12 January 2012; accepted 16 January 2012.