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Changes in Isoflavone Compositions of Soybean Foods during Cooking Process

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We evaluated the concentration and distribution of isoflavones in a total of 50 samples of soybean foods consumed daily in Japan by high-performance liquid chromatography (HPLC). A hierarchical cluster analysis using the measured isoflavone distribution classified these foods into 6 clusters. Experiments of heat processing representing standard commercial production processes of the foods clarified that each cluster was characterized by the effects of the heating method and temperature. Tofu, which is produced under mild heating conditions, showed similar isoflavone distribution to raw soybeans, having the highest proportion of 6″-*O*-malonyl-β-glucosides to total isoflavones, while soymilk, yuba, cooked soybean, and natto which are produced by comparatively stronger heat, showed a lower proportion of 6″-*O*-malonyl-β-glucosides and higher non-acylated-β-glucosides. In the production of kinako by roasting, the proportion of 6″-*O*-acetyl-β-glucosides, generated by decarboxylation of 6″-*O*-malonyl-β-glucosides, increased. Miso and soy sauce had an increased proportion of aglycons due to a lengthy fermentation period. Abura-age which is produced by frying tofu showed lower 6″-*O*-malonyl-β-glucosides than tofu, and higher 6″-*O*-acetyl-β-glucosides.

Keywords: isoflavones, soybean foods, cluster analysis, aglycons, β-glucosides, 6″-*O*-malonyl-β-glucosides, 6″-*O*-acetyl-β-glucosides

One feature of the traditional Japanese diet is the daily consumption of various kinds of soybean food, such as tofu, natto, cooked soybean, miso, and so on. Soybean is not only highly nutritious but also contains many elements useful to the biological defense mechanism (Messina & Messina, 1991). Isoflavone contained in soybean is also known as a phytoestrogen because of its estrogenic activities (Bickoff *et al*., 1962), and recently much attention has been focused on its physiological activities. Besides epidemiological studies (Adlercreutz *et al*., 1991; Lee *et al*., 1991), reports of animal (Baggott *et al*., 1990; Sharma *et al*., 1992) and *in vitro* (Adlercreutz *et al*., 1992; Wei *et al*., 1993) experiments suggest that increased soybean consumption results in a substantially lower incidence of breast and prostate cancer in East Asian regions including Japan compared to Western countries, due to the preventive effect of isoflavones contained in soybean against cancer. Furthermore, isoflavone prevents bone absorption (Ishida *et al*., 1998; Ishimi *et al*., 1999) and it is thought to have a preventive effect against osteoporosis.

Isoflavone contained in soybean consists of 9 glucosides and 3 aglycons (daidzein, genistein, and glycitein). The largest component, 6″-*O*-malonyl-β-glucosides (6″-*O*-malonyldaidzin, 6″-*O*-malonylgenistin, and 6″-*O*-malonylglycitin), becomes unstable upon heating and transforms to non-acylated-β-glucosides (hereafter β-glucosides), such as daidzin, genistin, and glycitin, or to 6″-*O*-acetyl-β-glucosides of 6″-*O*-acetyldaidzin, 6″-*O*-acetylgenistin, 6″-*O*-acetylglycitin. Consequently, heat applied during the processing of soybean products alters the distribution of isoflavones (Wang & Murphy, 1996; Coward *et al*., 1998). Furthermore, β-glucosides in fermented soybean foods are reported to be hydrolyzed and transformed to aglycons by β-glucosidase generated by microorganisms at fermentation (Ebata *et al*., 1972; Esaki *et al*., 1994). Also, we reported previously that 6″-*O*-succinyl-β-glucosides which do not exist in raw soybean are formed during the fermentation process of natto by *Bacillus subtilis* (*natto*) (Toda *et al*., 1999).

Although several reports describe changes in isoflavones during soybean food processing (Wang & Murphy, 1996; Coward *et al*., 1998), to the best of our knowledge no study has systematically determined the characteristics of isoflavone distribution according to the different kinds of soybean food, nor the relationship between these characteristics and processing methods, in particular heat application and water soaking. Therefore, in the present study, isoflavones from a total of 50 samples of commercial soybean foods were analyzed by high performance liquid chromatography (HPLC) in an attempt to classify the foods according to their distribution of isoflavones. The effects of soaking and heat processing on isoflavone distribution and transformation were also examined.

Materials and Methods

Commercial soybean foods and soybeans used for analysis of isoflavone The following items were bought at food stores in Kobe, Osaka, and Tokyo in 1999 and used as samples in the analysis of isoflavone: soymilk (aseptic packing), 4 samples; tofu, 6 samples; yuba, 4 samples; abura-age, 4 samples; cooked soybean (retort pouch), 6 samples; kinako, 5 samples; natto, 6 samples; miso, 5 samples; and soy sauce, 5 samples. Standard commercial production processes of these soybean foods are shown in Fig. 1 and 2. Five varieties of soybeans, all from 1998 crops, were used as samples in the analysis: 3 Japanese varieties (Toyomusume, Tachinagaha, Enrei), 1 USA variety (Vinton 81), and 1 Chinese variety (Touno).

Analysis of Isoflavone in commercial soybean foods and soybeans Analyses of isoflavone in soybean foods and soy-

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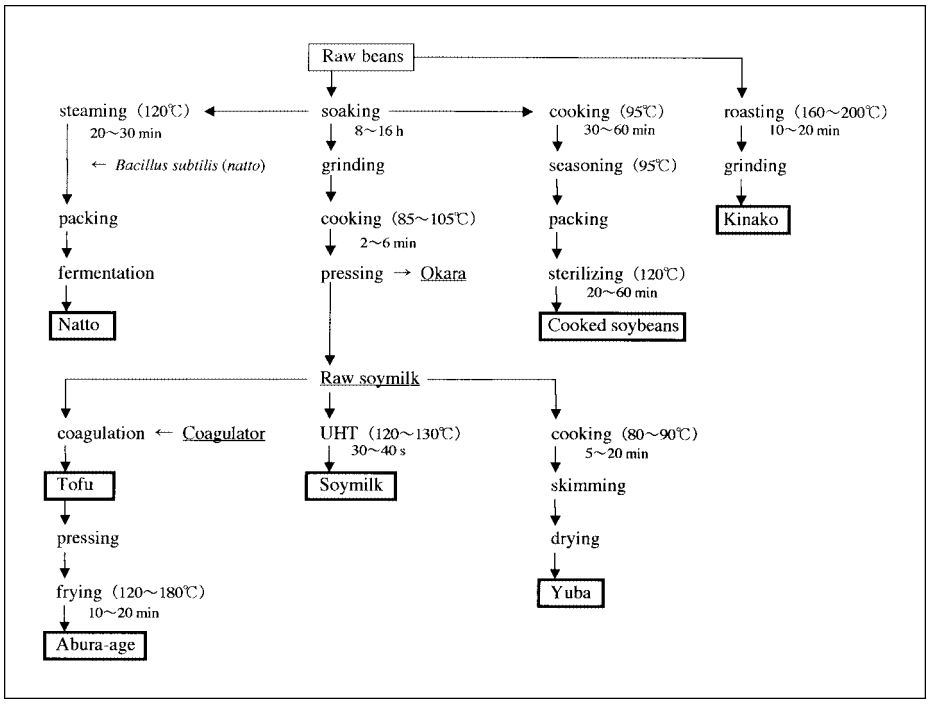

Fig. 1. Flow diagram illustrating the processing of soybeans to commercial soymilk, tofu, abura-age, yuba, natto, cooked soybeans and kinako.

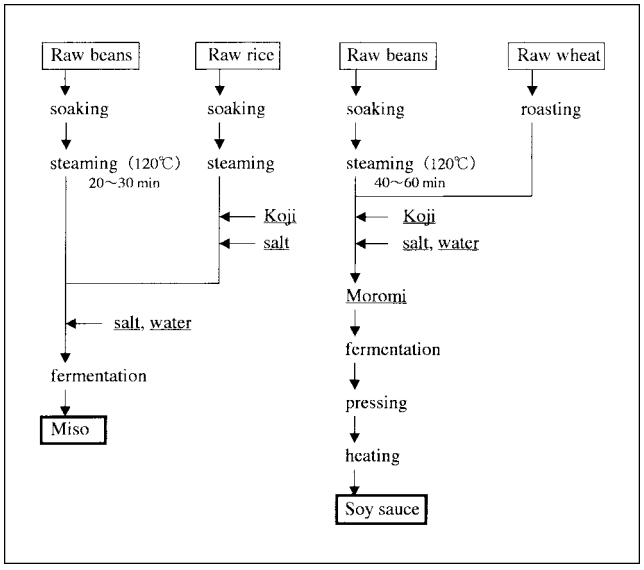

Fig. 2. Flow diagram illustrating the processing of soybeans to commercial miso and soy sauce.

beans themselves were carried out according to the method of Coward *et al.* (1998). Each sample was lyophilized by a freeze-dryer (FD-81, Tokyo Rika Kikai Co., Ltd., Tokyo) and then ground with a coffee mill, from which 0.5 g was extracted using 5 ml of 80% methanol with 1 mg fluorescein as an internal standard at 4°C for 24 h. Each extract was filtered by a 0.45 μm filter (Toyo Roshi Kaisha, Tokyo). Quantitative HPLC analysis was performed on a YMC-pack ODS-AM-303 column (250×4.6 mm), using a linear gradient of acetonitrile from 15 to 30% containing a fixed amount of acetic acid (0.1%) for 50 min. The solvent flow rate was 1 ml/min and absorption was measured at 254 nm. The results of measurements are given as means±standard deviations. Statistical significance of means was established at the $p<0.05$ level by ANOVA, followed by Fisher's PLSD method.

Standard isoflavones Daidzein, genistein, glycitein, daidzin, genistin, glycitin, 6″-*O*-malonyldaidzin, 6″-*O*-malonylgenistin, 6″-*O*-malonyleglycitin, 6″-*O*-acetyldaidzen, 6″-*O*-acetylgenistin, and 6″-*O*-acetylglycitin were isolated from soybeans using the method of Kudou *et al.* (1991).

6″-*O*-succinyldaidzin, 6″-*O*-succinylgenistin, and 6″-*O*-succinylglycitin were isolated from natto (Toda *et al.*, 1999).

Cluster analysis A hierarchical cluster analysis was performed using the distribution of aglycons, β-glucosides, 6″-*O*-malonyl-β-glucosides, and 6″-*O*-acetyl-β-glucosides contained in soybean foods and soybeans. The distance was calculated by a Euclidian formula and the clusters were fused by the nearest neighbor method. Cluster analysis was performed using a SPSS Statics Package (SPSS 10.0 J for Windows, Spss Inc, Chicago, IL).

Processing models of soybeans

Soaking Soybeans from the Hokkaido 1998 crop (Toyomusume) were soaked in distilled water (20°C, at 3 times their weight). After soaking for 0, 1, 2, 5, 10, and 15 h, the soaked soybeans and soaking liquid were lyophilized, ground to uniformity by a coffee mill, and prepared as samples for analysis. In order to confirm the effect of β-glucosidase in soybeans during soaking, another soaking model was conducted with 0.5% glucono-δ-lacton as the inhibitor of β-glucosidase (Reese & Parrish, 1971).

Heating processing—Boiling After soaking soybeans for 10 h under the same conditions as those for the water soaking model, the soaking liquid was drained using a 200 mesh sieve.

Distilled water at 10 times the wet weight was added to the soaked soybeans and these were then heated at 95°C for 0, 20, 40, 60 min. The heated soybeans and the liquid were then lyophilized and ground to uniformity using a coffee mill.

Heating processing—Steaming After soaking soybeans for 10 h under the same conditions as used for the water soaking model, the soaking liquid was drained using a 200 mesh sieve. The soaked soybeans were steamed in an autoclave (SS-325, Tomy Seiko Co., Ltd., Tokyo) at 120°C for 0, 5, 10, and 20 min. The soybeans and the drained liquid were lyophilized together, and then ground to uniformity using a coffee mill.

Heating processing—Roasting Soybeans from the Hokkaido 1998 crop (Toyomusume) were heated without prior soaking in water in an oven (DK-62, Yamato Scientific Co., Ltd., Tokyo) at 200°C for 0, 10, 20, and 30 min, and then ground using a coffee mill.

Isoflavone analysis of soybean samples from water soaking and heating models Extract of isoflavones and analysis by HPLC of soybean samples obtained by the water soaking and the heating models were conducted under the same conditions as those for the isoflavone analysis on soybeans and soybean foods. The measured values are indicated as means±standard deviations.

Results and Discussion

Analysis results of isoflavones contained in soybean foods and soybeans are presented in Table 1. The mean total isoflavones contained in the 5 varieties of soybeans was about 2500 μg/g. Most soybean isoflavones existed as glucosides; the highest proportion at more than 70% of the total was 6″-*O*-malonyl-β-glucosides, followed by β-glucosides at 20%, whereas 6″-*O*-acetyl-β-glucosides and aglycons occurred in only very small proportions. In contrast, the proportion of aglycons, β-glucosides, 6″-*O*-malonyl-β-glucosides, and 6″-*O*-acetyl-β-glucosides contained in commercial soybean foods varied distinctly according to the kind of food. We therefore inferred that distribution was affected by processing methods. 6″-*O*-succinyl-β-glucosides were detected only in natto. These are β-glucosides which have the succinyl group (-$COCH_2CH_2COOH$) bound to the OH group at the C-6 place of D-glucose, and we reported previously that 6″-*O*-succinyldaidzin, 6″-*O*-succinylgenistin, and 6″-*O*-succinylglycitin were generated during the course of fermentation by *Bacillus subtilis* (*natto*) (Toda *et al*., 1999).

However, when the distributions of total daidzein and its glucosides, the total of genistein and its glucosides, and the total of glycitein and its glucosides were reviewed, soybeans did not significantly differ from soybean foods, indicating that the structures of aglycons were not affected by processing methods.

A cluster analysis was then conducted based on the distribution of 12 isoflavones, with the exception of 6″-*O*-succinyl-β-glucosides since these are contained only in natto, and it was unnecessary to the investigation to check changes in isoflavone distribution originally contained in soybeans. The division into 6 clusters from A to F is clearly shown by the dendrogram in Fig. 3. Excluding 1 exceptional sample of soy milk and 1 of abura-age, all foods of the same kind were grouped in a cluster, enabling the recognition of characteristic patterns of isoflavone distribution according to each kind of food. Classifications were determined as follows: A, soybeans and tofu; B, soymilk and yuba; C, cooked soybean and natto; D, kinako; E, miso and soy sauce; and F, abura-age.

We believe that this classification into 6 clusters is characterized by the production process, particularly the heating condition, applied to each soybean food. Figures 1 and 2 show these standard production methods of each soybean food in order to illustrate differences in processing. The tofu production process

Table 1. Contents of isoflavone compounds in soybeans and commercial soybean foods[a)]

Sample		aglycon[b)] (%)			β-glucoside[b)] (%)			6″-*O*-malonyl-β-glucoside[b)] (%)		
		Dein	Gein	Glein	Din	Gin	Glin	Din	Gin	Glin
Soybean	n=5	0.8 ± 0.3^{C}	0.8 ± 0.3^{C}	0.0 ± 0.1^{D}	8.6 ± 3.1^{B}	9.2 ± 3.1^{D}	3.5 ± 1.8^{CD}	30.2 ± 4.4^{A}	37.1 ± 4.6^{A}	5.8 ± 2.1^{A}
Soymilk	n=4	1.8 ± 1.4^{C}	1.8 ± 1.8^{C}	0.2 ± 0.4^{CD}	23.6 ± 4.9^{A}	36.9 ± 10.1^{B}	2.4 ± 3.4^{CD}	10.9 ± 6.8^{C}	16.5 ± 12.4^{C}	1.3 ± 1.3^{C}
Tofu	n=6	2.7 ± 1.2^{C}	3.5 ± 1.4^{C}	0.1 ± 0.2^{D}	9.9 ± 2.1^{B}	13.2 ± 2.5^{D}	6.3 ± 1.3^{C}	22.5 ± 3.0^{B}	33.6 ± 5.2^{A}	6.9 ± 2.5^{A}
Yuba	n=4	4.8 ± 1.6^{C}	4.6 ± 1.9^{C}	2.4 ± 1.1^{B}	24.3 ± 5.0^{A}	35.6 ± 4.8^{B}	3.6 ± 0.5^{CD}	8.9 ± 2.7^{CD}	12.8 ± 4.5^{CD}	1.4 ± 0.4^{C}
Abura-age	n=4	2.8 ± 2.2^{C}	4.9 ± 3.4^{C}	nd^{D}	11.7 ± 4.2^{B}	10.7 ± 5.2^{D}	19.3 ± 13.9^{A}	6.2 ± 12.4^{CDE}	24.1 ± 2.2^{B}	nd^{C}
Cooked soybean	n=6	1.8 ± 2.1^{C}	3.6 ± 3.1^{C}	nd^{D}	25.1 ± 2.2^{A}	49.2 ± 6.8^{A}	3.8 ± 4.7^{CD}	4.4 ± 3.7^{DE}	3.5 ± 2.3^{E}	1.7 ± 1.9^{C}
Kinako	n=5	3.8 ± 1.6^{C}	4.9 ± 2.1^{C}	1.2 ± 1.6^{C}	20.8 ± 1.8^{A}	25.1 ± 4.1^{C}	1.6 ± 2.2^{CD}	0.3 ± 0.4^{E}	nd^{E}	0.5 ± 0.8^{C}
Natto	n=6	2.0 ± 0.7^{C}	2.7 ± 0.4^{C}	nd^{D}	24.6 ± 4.1^{A}	37.1 ± 4.9^{B}	12.4 ± 3.4^{B}	2.9 ± 3.2^{DE}	nd^{E}	3.8 ± 0.4^{B}
Miso	n=5	24.4 ± 7.9^{B}	38.7 ± 11.4^{A}	3.2 ± 0.8^{B}	7.5 ± 4.4^{B}	14.7 ± 5.9^{D}	0.7 ± 1.5^{CD}	2.6 ± 3.7^{DE}	6.1 ± 7.4^{DE}	nd^{C}
Soy sauce	n=5	45.1 ± 6.9^{A}	21.2 ± 6.1^{B}	6.6 ± 1.2^{A}	10.4 ± 6.7^{B}	13.1 ± 10.7^{D}	nd^{D}	nd^{E}	3.6 ± 5.0^{E}	nd^{C}

Sample		6″-*O*-acetyl-β-glucoside[b)] (%)			6″-*O*-succinyl-β-glucoside[b)] (%)			total	
		Din	Gin	Glin	Din	Gin	Glin	%	μg/g Wet Weight
Soybean	n=5	0.4 ± 0.3^{CD}	0.2 ± 0.3^{DE}	3.4 ± 0.4^{A}	nd^{B}	nd^{B}	nd^{B}	100	2516.6 ± 585.4^{B}
Soymilk	n=4	0.5 ± 0.4^{CD}	1.2 ± 0.2^{DE}	3.0 ± 3.4^{A}	nd^{B}	nd^{B}	nd^{B}	100	317.0 ± 132.4^{DE}
Tofu	n=6	0.5 ± 0.4^{CD}	0.9 ± 1.3^{DE}	nd^{B}	nd^{B}	nd^{B}	nd^{B}	100	459.9 ± 46.8^{D}
Yuba	n=4	0.7 ± 0.2^{CD}	0.2 ± 0.4^{DE}	0.7 ± 0.8^{B}	nd^{B}	nd^{B}	nd^{B}	100	2800.5 ± 616.5^{A}
Abura-age	n=4	6.8 ± 2.9^{B}	13.5 ± 6.0^{B}	nd^{B}	nd^{B}	nd^{B}	nd^{B}	100	646.1 ± 109.0^{D}
Cooked soybean	n=6	1.7 ± 0.9^{C}	5.2 ± 0.8^{C}	nd^{B}	nd^{B}	nd^{B}	nd^{B}	100	605.1 ± 215.4^{D}
Kinako	n=5	15.9 ± 2.9^{A}	24.7 ± 2.9^{A}	1.2 ± 1.6^{B}	nd^{B}	nd^{B}	nd^{B}	100	2476.4 ± 349.2^{AB}
Natto	n=6	0.7 ± 0.6^{CD}	2.6 ± 0.8^{D}	nd^{B}	4.1 ± 1.9^{A}	6.6 ± 5.1^{A}	0.3 ± 0.8^{A}	100	1642.0 ± 455.7^{C}
Miso	n=5	nd^{D}	2.1 ± 0.2^{DE}	nd^{B}	nd^{B}	nd^{B}	nd^{B}	100	303.8 ± 104.3^{DE}
Soy sauce	n=5	nd^{D}	nd^{E}	nd^{B}	nd^{B}	nd^{B}	nd^{B}	100	13.7 ± 3.1^{E}

[a)] Values represent the mean±standard deviation. Percentages were calculated on mole basis.

[b)] Dein=daidzein; Gein=genistein; Glein=glycitein; Din=daidzin; Gin=genistin; Glin=glycitin. nd=not detectable. Values in a column with different superscripts were significantly different ($p<0.05$).

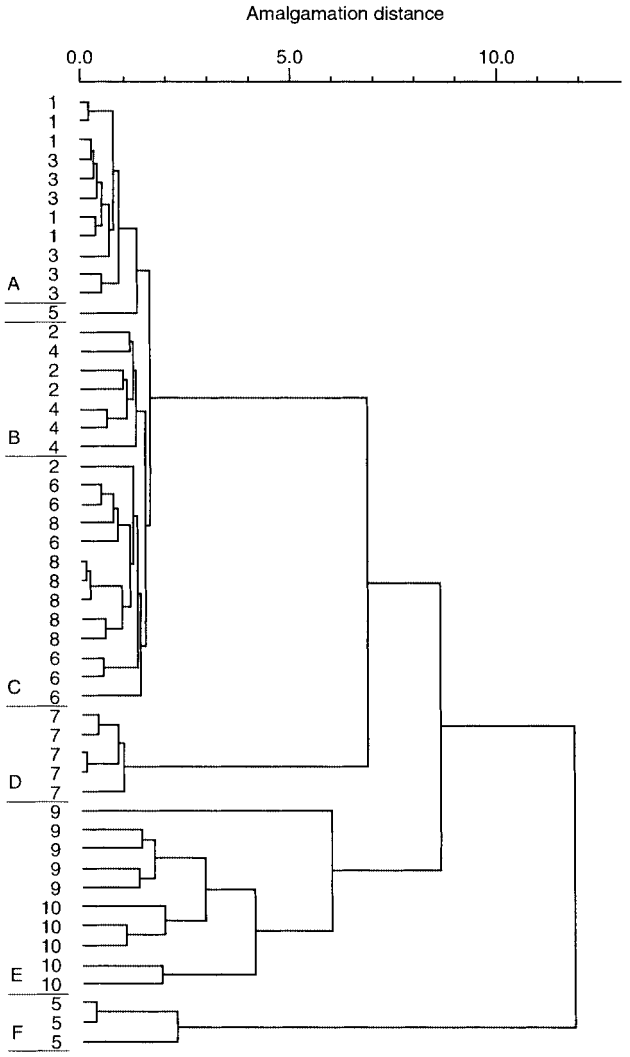

Fig. 3. Dendrogram of soybeans and commercial soybean foods connected on the basis of the distribution of isoflavone compounds. 1, soybean; 2, soymilk; 3, tofu; 4, yuba; 5, aburaage; 6, cooked soybean; 7, kinako; 8, natto; 9, miso; 10, soy sauce.

involves heating at 85–105°C for 2–6 min. This heating condition is mild compared to the heating conditions of the other commercial soybean foods. Ultra high-temperature (UHT) sterilization is applied to the production of most commercial soymilk. Yuba is produced by skimming and drying the film formed on the surface of heated soymilk. Cooked soybean is retort sterilized, while natto is strongly heated by pressurized steaming. Kinako is produced differently from other soybean foods, by dry roasting at a high temperature without prior soaking in water. Miso and soy sauce are also subjected to pressurized steaming like natto, but they are fermented by bacteria afterwards for a lengthy period. The production of abura-age differs considerably from other foods since it is fried in oil at 120–180°C.

To elucidate the effect of heating on the altered distribution of aglycons, β-glucosides, 6″-*O*-malonyl-β-glucosides, and 6″-*O*-acetyl-β-glucosides in soybean foods during each production process, heating model tests using soybeans were carried out. Figures 1 and 2 show, except for kinako, that the processing of soybean foods includes soaking soybeans in water first, followed by heating. Therefore, changes occurring during water soaking

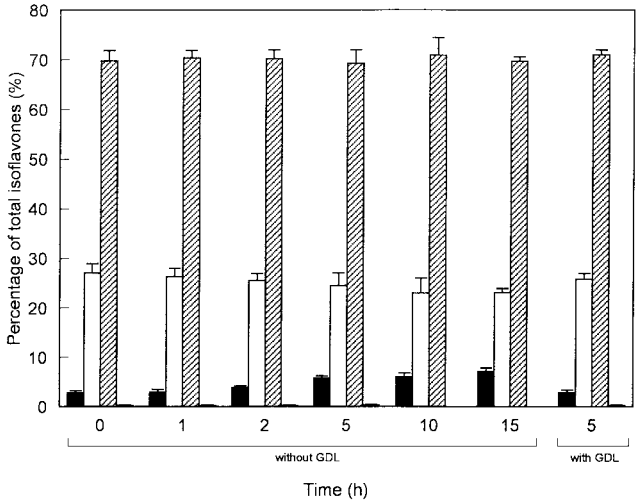

Fig. 4. Changes in isoflavone compounds in soaking soybeans with and without glucono-δ-lactone (GDL, 0.5%) at 20°C. Mean±SD. n=4. ■, aglycons; □, β-glucosides; ▨, 6″-*O*-malonyl-β-glucosides; ▩, 6″-*O*-acetyl-β-glucosides.

were investigated first. Figure 4 shows that as soaking time increases, the proportion of aglycon increases slightly and in parallel, whereas β-glucosides decrease. Matsuura *et al.* (1989) reported that aglycons of soybeans increased during water soaking due to the activity of β-glucosidase. To confirm that the slight increase of aglycons in this experiment was also due to β-glucosidase, the changes were observed after the addition of glucono-δ-lacton, its inhibitor, to the soaking soybeans. The addition of glucono-δ-lacton restricted the increase in aglycons, suggesting that the increase in the proportion of aglycons during water soaking was attributable to the activity of β-glucosidase contained in the soybeans. However, as water soaking normally occurs at below 20°C, the activity of β-glucosidase is very weak (Matsuura *et al.*, 1989), and thus has only a slight effect on changes in overall isoflavone distribution.

Figure 5 (a and b) show changes in isoflavones when water-soaked soybeans were subjected to boiling at 95°C and steaming at 120°C. In both cases, 6″-*O*-malonyl-β-glucosides decreased and β-glucosides increased according to the processing time. 6″-*O*-malonyl-β-glucosides are unstable upon heating, and previous reports have described significant differences in quantitative values obtained from extraction by heating and at room temperature (Kudou *et al.*, 1991). In the present study at 95°C, the majority of 6″-*O*-malonyl-β-glucosides transformed to β-glucosides upon heating, whereas, although 6″-*O*-acetyl-β-glucosides also increased slightly, only a very small amount was changed to 6″-*O*-acetyl-β-glucosides. These tendencies were more significant upon processing at 120°C. As shown in Table 1, the proportion of 6″-*O*-malonyl-β-glucosides to total isoflavones was 73% and 63% for soybeans and tofu, respectively, as opposed to 29% in soy milk, 23% in yuba, and only a small percentage in cooked soybean and natto which were subjected to stronger heating conditions. In contrast to the decrease in the proportion of 6″-*O*-malonyl-β-glucosides, that of β-glucosides increased. Since these findings are consistent with the results from the experimental heating procedure, the distribution of isoflavones in these foods is probably due to heating during processing.

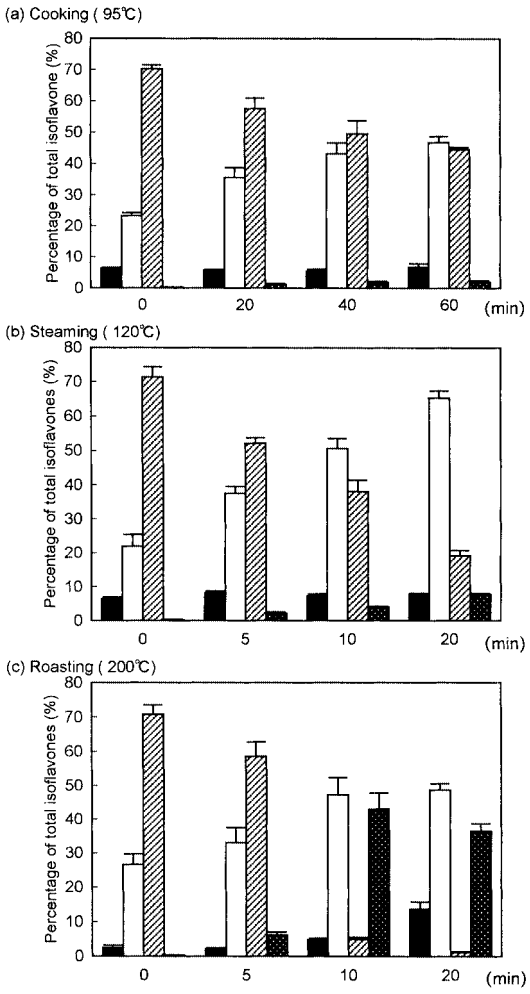

Fig. 5. Changes in isoflavone compounds in cooking, steaming and roasting soybeans. Mean±S.D. n=4. ■, aglycons; □, β-glucosides; ▨, 6″-*O*-malonyl-β-glucosides; ▩, 6″-*O*-acetyl-β-glucosides.

Like cooked soybean and natto, miso and soy sauce are also treated by steaming, and thus the proportion of 6″-*O*-malonyl-β-glucosides is only a small percentage. However, the proportion of aglycons is higher than that of β-glucosides in miso and soy sauce (Fig. 3), and therefore these were classified as a separate cluster (Fig. 3). During the fermentation of miso and soy sauce, β-glucosides are reported to be hydrolyzed to aglycons by β-glucosidase generated by bacteria, *Aspergillus* strain (Esaki *et al.*, 1994). Although natto formed by *Bacillus subtilis* (*natto*) is a fermented soybean food, aglycons increased very little, the proportion of β-glucosides was very high at 74%, and the distribution was similar to that of cooked soybean.

Figure 5 (c) shows the change in isoflavones upon roasting soybeans at 200°C without prior soaking of them in water. During the first 10 min of the process, 6″-*O*-acetyl-β-glucosides increased drastically while 6″-*O*-malonyl-β-glucosides decreased drastically. Continued roasting showed a slightly decreased proportion of the 6″-*O*-acetyl-β-glucosides. We believe that most 6″-*O*-malonyl-β-glucosiedes were decarboxylated and changed to 6″-*O*-acetyl-β-glucosides when roasted at a high temperature. β-Glucosides and aglycons also increased gradually over time. In the cluster analysis, kinako formed a single cluster, showing only a slight similarity to other soybean foods (Fig. 3), which is regarded as probably the result of the very high proportion of 6″-*O*-accetyl-β-glucosides caused by roasting compared with other soybean foods.

Abura-age which is produced by frying tofu in oil also formed one independent cluster (Fig. 3). In comparison with tofu, heating in oil at a high temperature to produce abura-age resulted in a smaller proportion of 6″-*O*-malonyl-β-glucosides and a higher proportion of 6″-*O*-acetyl-β-glucosides. We assumed this was the reason for the formation of an independent cluster.

Izumi *et al.* (2000) reported that some isoflavone aglycons were absorbed faster and in greater amounts than their glucosides in humans. Though information on the absorption of the other derivatives (6″-*O*-malonyl-β-glucosides and 6″-*O*-acetyl-β-glucosides) has not yet been obtained, the chemical structure of isoflavone derivatives is presumed to have significant influence on their bioavailability. Therefore, the chemical structure of the derivatives contained in processing soybean foods is important in evaluating their physical roles.

The findings of the present study confirm that the isoflavones contained in soybeans are mainly 6″-*O*-malonyl-β-glucosides, but are transformed to β-glucosides, 6″-*O*-acetyl-β-glucosides, and aglycons during soaking and heat processing of soybean foods. Our findings of characteristic patterns in the distribution of isoflavones according to each kind of processed food reveals that such changes are significantly affected by the method, temperatures and duration of heating.

References

Adlercreutz, H., Honjo, H., Higashi, A., Fotsis, T., Hämäläinen, E., Hasegawa, T. and Okada, H. (1991). Urinary excretion of lignans and isoflavonoid phytoestrogens in Japanese men and women consuming a traditional Japanese diet. *Am. J. Clin. Nutr.*, **54**, 1093–1100.

Adlercreutz, H., Mousavi, Y., Clark, J., Höckerstedt, K., Hämäläinen, E., Wähälä, K., Mäkelä, T and Hase, T. (1992). Dietary phytoestrogens and cancer: *in vitro* and *in vivo* studies. *J. Steroid Biochem. Mol. Biol.*, **41**, 331–337.

Baggott, J.E., Ha, T., Vaughn, W.H., Juliana, M.M., Hardin, J.M. and Grubbs, C.J. (1990). Effect of miso (Japanese soybean paste) and NaCl on DMBA-induced rat mammary tumors. *Nutr. Cancer*, **14**, 103–109.

Bickoff, E.M., Livingston, A.L., Hendrickson, A.P. and Booth, A.N. (1962). Relative potencies of several estrogen-like compounds found in forages. *J. Agric. Food Chem.*, **10**, 410–412.

Coward, L., Smith, M., Kirk, M. and Barnes, S. (1998). Chemical modification of isoflavones in soyfoods during cooking and processing. *Am. J. Clin. Nutr.*, **68**, 1486S-1491S.

Ebata, J., Fukuda, Y., Hirai, K. and Murata, K. (1972). β-glucosidase involved in the antioxidant formation in tempeh, fermented soybeans. *Nippon Nougei Kagaku Kaishi*, **46**, 323–329 (in Japanese).

Esaki, H., Onozaki, H. and Osawa, T. (1994). Antioxidative Activity of Fermented Soybean Products. In "Food Phytochemicals for Cancer Prevention 1," American Chemical Society, Washington D.C., pp. 353–360.

Ishida, H., Uesugi, T., Hirai, K., Toda, T., Nukaya, H., Yokotsuka, K. and Tsuji, K. (1998). Preventive effects of the plant isoflavones, daidzin and genistin, on bone loss in ovariectomized rats fed a calcium-deficient diet. *Biol. Pharm. Bull.*, **21**, 62–66.

Ishimi, Y., Miyaura, C., Ohmura, M., Onoe, Y., Sato, T., Uchiyama, Y., Ito, M., Wang, X., Suda, T. and Ikegami, S. (1999). Selective effects of genistein, a soybean isoflavone, on B-lymphopoiesis and bone loss caused by estrogen deficiency. *Endocrinology*, **140**, 1893–1900.

Izumi, T., Piskula M.K., Osawa, S., Obata, A., Tobe, K., Saito, M., Kataoka, S., Kubota, Y. and Kikuchi, M. (2000). Soy isoflavone

aglycones are absorbed faster and in higher amounts than their glucosides in humans. *J. Nutr.*, **130**, 1695–1699.

Kudou, S., Fleury, Y., Welti, D., Magnolato, D., Uchida, T., Kitamura, K. and Okubo, K. (1991). Malonyl isoflavone glycosides in soybean seeds (*Glycine max* MERRILL). *Agric. Biol. Chem.*, **55**, 2227–2233.

Lee, H.P., Gourley, L., Duffey, S.W., Esteve, J., Lee, J. and Day, N.E. (1991). Dietary effects on breast-cancer risk in Singapore. *Lancet*, **337**, 1197–1200.

Matsuura, M., Obata, A. and Fukushima, D. (1989). Objectionable flavor of soy milk developed during the soaking of soybeans and its control. *J. Food Sci.*, **54**, 602–605.

Messina, M. and Messina, V. (1991). Increasing use of soybeans and their potential role in cancer prevention. *J. Am. Diet. Assoc.*, **91**, 836–840.

Reese, E.T. and Parrish, F.W. (1971). Nojirimycin and D-glucono-1, 5-lactone as inhibitors of carbohydrases. *Carbohydr. Res.*, **18**, 381–388.

Sharma, O.P., Adlercreutz, H., Strandberg, J.D., Zirkin, B.R., Coffey, D.S. and Ewing, L.L. (1992). Soy of dietary source plays a preventive role against the pathogenesis of prostatitis in rats. *J. Steroid Biochem. Mol. Biol.*, **43**, 557–564.

Toda, T., Uesugi, T., Hirai, K., Nukaya, H., Tsuji, K. and Ishida, H. (1999). New 6″-*O*-acyl isoflavone glycosides from soybeans fermented with *Bacillus subtilis* (*natto*). 1. 6″-*O*-succinylated isoflavone glycosides and their preventive effects on bone loss in ovariectomized rats fed a calcium-deficient diet. *Biol. Pharm. Bull.*, **22**, 1193–1201.

Wang, H-J. and Murphy, P.A. (1996). Mass balance study of isoflavones during soybean processing. *J. Agric. Food Chem.*, **44**, 2377–2383.

Wei, H., Wei, L., Frenkel, K., Bowen, R. and Barnes, S. (1993). Inhibition of tumor promoter-induced hydrogen peroxide formation *in vitro* and *in vivo* by genistein. *Nutr. Cancer*, **20**, 1–12.