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Effect of soybean varieties on the content and composition of isoflavone in rice-koji miso

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Abstract

To clarify the effect of soybean varieties on isoflavone, a useful component for human health, in soybean products, we investigated changes in the isoflavone content and composition in rice-koji miso, after fermentation/aging for 6 or 12 months using varieties of soybeans (Tohoku-126, Tohoku-135, Tohoku-139, Suzuyutaka and Chinese soybeans), by high performance liquid chromatography. In soybeans, the total isoflavone content in Tohoku-126 was 444 mg/100 g, which was 1.2–2.0 times the content in the other soybean varieties. The malonyl glycosides and aglycones in soybeans accounted for more than 60% and only a few percent, respectively. As for rice-koji miso, the total isoflavone and aglycone contents were the highest in miso prepared from Tohoku-126. The ratios of glycosides to aglycones (80.1–92.6%) in miso were higher than those in the original soybeans. The time course of the isoflavone composition during the fermentation/aging process of rice-koji miso indicated that glycosides decreased from 86.4% to 44.9% after 6 months but aglycones increased from 9.6% to 53.3%.

Keywords: Soybean; Miso; Isoflavone

1. Introduction

Soybeans are massively consumed in Asian nations. In Japan, soybeans are used for various soybean products including miso (soybean paste), soybean curd and fermented soybean. Soybeans contain various physiologically active substances and inhibit carcinogenesis (Kennedy, 1995). In animal studies, soy protein isolate and miso have been shown to inhibit chemically induced cancers (Constantinou et al., 2001; Gotoh et al., 1998; Hawrylewicz, Zapata, & Blair, 1995). Epidemiological studies have suggested that a high soybean intake is related to low incidence of breast cancer and prostatic cancer in Asians (Adlercreutz et al., 1991; Herman et al., 1995). The above information suggests that active ingestion of soybeans and soybean products is useful for human health.

A useful component in soybeans includes isoflavone, which has different types of aglycone (daidzein, genistein and glycitein), their glycoside forms (daidzin, genistin and glycitin) and their malonyl glycoside and acetyl glycoside forms (Kudou et al., 1991; Wang & Murphy, 1994). Isoflavone not only inhibits breast cancer and prostatic cancer but also exhibits many physiological actions, such

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as estrogen action, improvement of menopause symptoms and prevention of osteoporosis and circulatory disorders (Anthony, Clarkson, & Williams, 1998; Bingham, Atkinson, Liggins, Bluck, & Coward, 1998; Evans, Griffiths, & Morton, 1995; Maskarinec, Williams, Inouye, Stanczyk, & Franke, 2002; Peterson et al., 1998; Potter et al., 1998; Wu et al., 1996). To utilize such physiological functions, the amount of isoflavone ingestion is an important factor. In humans, ingestion of 1.5–2.0 mg/kg/day of isoflavone has been suggested to inhibit carcinogenesis (Hendrich, Lee, Xu, Wang, & Murphy, 1994). For efficient exertion of the effect of isoflavone, intestinal absorbability is also an important factor. It has been shown that aglycone types of isoflavone are more absorbable than glycoside types in humans (Izumi et al., 2000). It has also been reported that physiological function of isoflavone varies among isoflavones. For example, regarding the anticancer action of isoflavone, genistein has been mostly shown to markedly inhibit prostatic cancer cell growth (Onozawa et al., 1998). Thus, the contents and compositions of isoflavone in soybeans and soybean products are important factors of their health effects in humans.

However, in soybean products, the content and composition of isoflavone change, due to the processing method and production process. For example, malonyl glycosides account for a high ratio in soybean curd, but aglycones are the main isoflavone in fermented soybean products (Wang & Murphy, 1994). Soaking, heat-processing, and coagulation in the production process of soybean curd have been reported to decrease the isoflavone content (Wang & Murphy, 1996). These reports suggest that the differences in the processing method and production process affect the function of soybean products. Therefore, it is important to clarify changes in the content and composition of isoflavone in soybean products and during the production process.

The objective of this study was to investigate the relationship between varieties of soybeans and the content and composition of isoflavone in soybean products and to clarify suitability of the soybean varieties for processing. In this study, using five varieties of soybeans, we prepared miso, a Japanese traditional fermented food, and measured the content and composition of isoflavone, in the fermentation/aging step and in the product, by high performance liquid chromatography.

2. Materials and methods

2.1. Materials

For soybeans, high-isoflavone soybeans (Tohoku-126), lipoxygenase-deleted soybeans (Tohoku-135), soybeans recommended by Fukushima prefecture (Suzuyutaka), low-allergen soybeans (Tohoku-139) (all these are produced in Fukushima prefecture) and Chinese soybeans, five varieties in total, were used. For rice, Koshihikari produced in Fukushima prefecture was used. For salt, common commercial salt was used.

2.2. Preparation of rice-koji miso

The brewing process is outlined in Fig. 1. The rice-koji miso was prepared using 80% koji (rice weight/soybean weight × 100). The nominal water content and salt concentration were 48% and 12.5%, respectively. The ingredients for the brewing process were prepared as follows: for the four varieties of soybeans produced in Fukushima prefecture, 17.6 kg of steamed rice with seed *Aspergillus oryzae*, 8.3 kg of salt, 689 ml of cultured yeast suspension and 2.3 kg of broth were added to 40.0 kg of boiled and steamed soybeans. For Chinese soybeans, 17.6 kg of steamed rice with seed *A. oryzae*, 8.5 kg of salt, 708 ml of cultured yeast suspension and 4.1 kg of broth were added to 40.0 kg of boiled and steamed soybeans. Yeast was added at 1.2×10^6 cells/g total ingredient. The miso was fermented and aged for 6 or 12 months by natural fermentation.

2.3. Chemical analyses of soybeans and miso

The chemical analysis of miso was performed according to the Official Method of Miso Analysis (Institute of Miso Technologists, 1968). The contents of water and protein in the soybeans and the brightness (*Y*%) of the boiled and steamed soybeans were also measured by the Official Method.

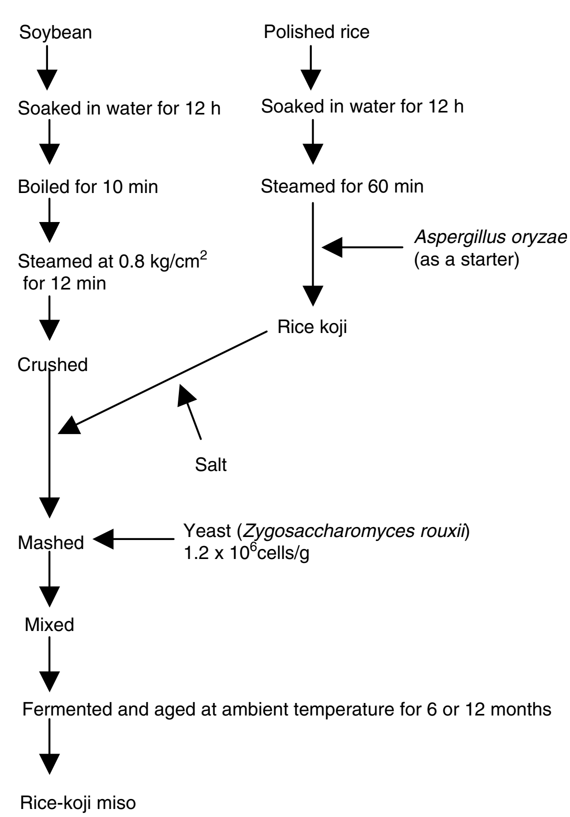

Fig. 1. Schematic representation of brewing process of rice-koji miso.

2.4. Measurement of isoflavones in soybeans and miso

Isoflavones in the soybeans and miso were measured by high performance liquid chromatography (HPLC) as previously reported (Yamabe, Kobayashi, Kaneko, Yaghi, & Takita, 2004). In measurement of glycoside (I) and aglycone, a specified amount of the sample was accurately weighed, combined with 10-fold amounts of 0.1% acetic acid–80% ethanol, and extracted by stirring at room temperature for 2 h. As for malonyl glycosides, the sample was combined with 0.1% acetic acid–80% ethanol, and hydrolyzed at 80 °C for 15 min to extract it as glycoside (II). The both extracts were centrifuged (3000 rpm, 10 min) and the supernatants were filtered through a microfilter and each filtrate (10 μl) was injected. For the instrument and the column, a HITACHI L-7 series (detector: UV 260 nm) (Hitachi Ltd., Tokyo, Japan) and LiChrosorb PR-18 (4.0 i.d. × 250 mm) (GL Science Inc., Tokyo, Japan), respectively, were used. For the mobile phase, 20 mM sodium phosphate buffer (pH 3.0) as Solution A and acetonitrile as Solution B were used (Solution B was 10% at initiation and 50% at 30 min). The flow rate and the column temperature were 1.0 ml/min and 30 °C, respectively. The content of malonyl glycosides was obtained by subtracting the content of glycoside (I) from that of glycoside (II).

3. Results and discussion

Table 1 shows the analytical values of water, protein and brightness (*Y*%), which is the colour scale of soybeans, of Tohoku-126, Tohoku-135, Suzuyutaka, Tohoku-139 and Chinese soybeans. The water content was 11.6–12.5%, showing almost no difference among the varieties. The protein content was 32.3–34.8%, showing no major difference among the varieties. The *Y*% of boiled and steamed soybeans was 40.8–43.1%, showing no marked difference among the varieties.

Table 2 shows the chemical components, pH and the colour scale (*Y*%, hue (*x*) and saturation (*y*)) of the miso made from Tohoku-126, Tohoku-135, Suzuyutaka, Tohoku-139 and Chinese soybeans. Overall, the water content was 43.7–46.1%; NaCl content, 12.0–12.2%; total nitrogen (T-N), 1.84–2.01%; formol nitrogen (F-N), 0.44–0.45%; degradation rate of T-N (F-N/T-N × 100), 22.3–23.9%; ethanol, 0.72–1.01%; and pH, 5.27–5.31, showing no major differences among the varieties. Hue (*x*) was 0.432–0.452, and saturation (*y*) was 0.400–0.411, showing almost no influence of the varieties. *Y*% was 18.9–24.2%, showing slight differences among the varieties. The brightness of miso was lower than that of the original soybeans. This is probably attributable to melanoidin produced by amino-carbonyl reaction during fermentation/aging of miso.

Soybeans contain daidzin, genistin, and glycitin as isoflavone glycosides (Kudou et al., 1991). In this study, abundant daidzin and genistin, their malonyl glycosides, and their aglycones, daidzein and genistein, were investigated. Table 3 shows the content and composition of isoflavone in the soybean varieties. The total isoflavone content ranged from 221 to 444 mg/100 g. The isoflavone content in Tohoku-126 was 1.2–2.0 times higher than that in the other varieties. Regarding the composition of isoflavone in soybeans, malonyl glycosides were the major components (Kudou et al., 1991), accounting for 57.2–72.2%. The ratio of malonyl glycosides was highest in Tohoku-139 and lowest in Chinese soybeans. Glycosides accounted for 26.7–40.6%, and the ratio was highest in Chinese soybeans and lowest in Tohoku-139. Malonyl glycosides and glycosides accounted for 97.2–98.9% of isoflavone in the soybeans. In contrast, the ratio of aglycones was low (1.1–2.8%) in all varieties. On comparison of daidzein series (malonyl daidzin + daidzin + daidzein) and genistein series (malonyl genistin + genistin + genistein), the daidzein and genistein series accounted for 42.1–49.9% and 50.2–57.9%, respectively, showing a slightly higher ratio of the genistein series. This trend was marked in Suzuyutaka, Tohoku-139 and Chinese soybeans.

Table 4 shows the content of each isoflavone in miso made from the five varieties of soybeans by fermentation/aging for 6 months. The total isoflavone content was 54.0–61.0 mg/100 g in miso made from Tohoku-135, Suzuyutaka, Tohoku-139, and Chinese soybeans. In contrast, in miso made from Tohoku-126, the variety with highest isoflavone content, the isoflavone content was 137 mg/100 g, which was 2.2–2.5 times higher than the contents in the other miso. The isoflavone content in miso partly reflected that in the original soybeans. Regarding the composition of isoflavone in miso, malonyl glycosides that accounted for 57.2–72.2% in the soybeans markedly decreased to 7.4–19.9%, and glycosides and aglycones increased. Aglycones accounted for only 1.1–2.8% in the soybeans, but markedly increased to 34.1–53.2% in miso, and the ratio was highest in the miso made from Chinese soybeans, in which the ratio of malonyl glycosides was low and the ratio of glycosides was high. Increases in glycosides and aglycones in miso may have been due to conversion of malonyl glycosides in soybeans to glycosides during the boiling and steaming processes, and the glycosides may have been hydrolyzed to aglycones by β-glucosidase produced by rice-koji during fermentation/aging. The ratio of aglycones was high in miso made from Chinese soybeans, and this may have been due to a high content

Table 1
Chemical analysis of various soybeans

Varieties	Moisture[a]	Protein[a]	*Y* (%)
Tohoku-126	11.6	34.6	41.8
Tohoku-135	12.0	32.3	43.1
Suzuyutaka	12.3	33.2	41.2
Tohoku-139	11.9	34.8	42.9
Chinese soybeans	12.5	32.8	40.8

Abbreviations: *Y* (%), brightness.
[a] Weight percent.

Table 2
Chemical analysis of various miso

Raw soybeans	Proportion of chemical components[a]						pH	Colour		
	Moisture	NaCl	T-N	F-N	F-N/T-N	Ethanol		*x*	*y*	*Y* (%)
Tohoku-126	43.7	12.2	1.97	0.44	22.3	0.84	5.29	0.436	0.400	21.2
Tohoku-135	46.1	12.1	1.84	0.44	23.9	0.91	5.27	0.452	0.407	18.9
Suzuyutaka	45.6	12.1	1.90	0.45	23.7	0.90	5.27	0.435	0.403	22.4
Tohoku-139	44.6	12.2	2.01	0.45	22.4	0.72	5.29	0.432	0.403	24.2
Chinese soybeans	46.1	12.0	1.86	0.44	23.7	1.01	5.31	0.440	0.411	23.0

Abbreviations: T-N, total nitrogen; F-N, formol nitrogen; F-N/T-N, degradation ratio of total nitrogen; *Y* (%), brightness; *x*, hue; *y*, saturation.
[a] Weight percent.

Table 3
Isoflavone contents in soybeans used in this study

Raw soybeans	Isoflavone (mg/100 g)[a]						Total
	Malonyl daidzin	Malonyl genistin	Daidzin	Genistin	Daidzein	Genistein	
Tohoku-126	150 (33.8)	161 (36.3)	56.5 (12.7)	66.0 (14.9)	5.3 (1.2)	5.3 (1.2)	444 (100.0)
Tohoku-135	134 (36.5)	128 (35.1)	45.9 (12.6)	53.1 (14.5)	2.9 (0.8)	2.1 (0.6)	366 (100.0)
Suzuyutaka	73.4 (28.5)	96.6 (37.5)	32.1 (12.5)	48.3 (18.7)	3.7 (1.4)	3.6 (1.4)	258 (100.0)
Tohoku-139	71.4 (32.3)	88.4 (39.9)	23.5 (10.6)	35.6 (16.1)	1.3 (0.6)	1.1 (0.5)	221 (100.0)
Chinese soybeans	78.9 (24.3)	106.7 (32.9)	54.0 (16.7)	77.6 (23.9)	3.7 (1.1)	3.5 (1.1)	324 (100.0)

[a] The numbers in parentheses show the ratio of each isoflavone to total isoflavone.

Table 4
Isoflavone contents in miso prepared in this study and commercial miso products

Raw soybeans	Isoflavone (mg/100 g)[a]						Total
	Malonyl daidzin	Malonyl genistin	Daidzin	Genistin	Daidzein	Genistein	
rice-koji miso							
Tohoku-126	10.1 (7.4)	17.1 (12.5)	26.7 (19.5)	36.3 (26.5)	25.9 (18.9)	20.8 (15.2)	137.0 (100.0)
Tohoku-135	1.8 (3.4)	4.2 (7.9)	8.2 (15.2)	13.9 (25.8)	13.0 (24.1)	12.8 (23.7)	54.0 (100.0)
Suzuyutaka	1.3 (2.4)	2.7 (5.0)	8.3 (15.4)	18.1 (33.2)	11.0 (20.3)	12.9 (23.8)	54.3 (100.0)
Tohoku-139	4.5 (8.1)	3.3 (6.0)	8.6 (15.5)	17.7 (31.8)	9.9 (17.7)	11.6 (20.9)	55.7 (100.0)
Chinese soybeans	4.9 (8.1)	2.8 (4.7)	6.3 (10.3)	14.5 (23.8)	14.6 (23.9)	17.9 (29.3)	61.0 (100.0)
Commercial miso							
rice-koji miso	1.9 (2.5)	2.7 (3.5)	18.3 (23.6)	26.7 (34.5)	12.4 (16.0)	15.5 (20.0)	77.5 (100.0)
barley-koji miso	0.6 (2.0)	1.2 (4.0)	3.2 (10.8)	4.1 (13.8)	9.2 (31.0)	11.4 (38.4)	29.7 (100.0)
soybean-koji miso	tr (0.0)	0.1 (0.1)	3.2 (3.6)	3.0 (3.4)	36.4 (40.7)	46.8 (52.3)	89.5 (100.0)

Abbreviation: tr, trace.
[a] The numbers in parentheses show the ratio of each isoflavone to total isoflavone.

of glycosides in the soybeans (Table 3), on which β-glucosidase may have readily acted. On comparison of the ratio of daidzein to the total of daidzin and daidzein and the ratio of genistein to the total of genistin and genistein, the ratio of daidzein was higher in all of the miso. Considering that the content of the genistein series was higher in the soybeans, β-glycosidase in the miso prepared may have preferably acted on daidzin.

The content and composition of isoflavone in the miso prepared in this study were compared with those in commercial rice-koji miso, barley-koji miso and soybean-koji miso (Table 4). The total isoflavone contents in the commercial rice-koji miso, barley-koji miso and soybean-koji miso were 77.5, 29.7 and 89.5 mg/100 g, respectively. As for the isoflavone composition in the commercial miso, the ratios of aglycones and glycosides were high, as in the rice-koji miso prepared in this study, and the total of the two forms accounted for more than 94.0%. This trend was marked in the soybean-koji miso, in which 99.9% of isoflavone consisted of glycosides and aglycones. The ratios of glycosides and aglycones varied among the miso types. The ratio of glycosides was high in rice-koji miso (58.1%), while the ratio of aglycones was high in barley-koji miso (69.4%) and soybean-koji miso (93.0%). Since the duration of fermentation/aging of soybean-koji miso was 2–3 years, longer than that of the other miso types, the action of β-glycosidase may have been sufficient, increasing aglycones (Murakami, Asakawa, Terao, & Matsushita, 1984). Because the aglycon form of isoflavone has been reported to be more absorbable (Izumi et al., 2000), soybean-koji miso, which contains abundant isoflavone, particularly aglycone, may be the best miso for efficient ingestion of isoflavone. On comparison of the rice-koji miso prepared in this study and the commercial miso, the amounts of total isoflavone and aglycon in the rice-koji miso made from Tohoku-126 were higher than those in the commercial rice-koji miso and barley-koji miso. Although the aglycone amount in the rice-koji miso made from Tohoku-126 was lower than that in the commercial soybean-koji miso, the total isoflavone content was higher. Based on these findings, miso made from Tohoku-126 is a superior source of isoflavone in quality and quantity.

Among the five soybean varieties, Chinese soybeans had the lowest ratio of malonyl glycosides and the highest ratio of glycosides. Thus, using the rice-koji miso made from Chinese soybeans, time-course changes in the isoflavone composition during fermentation/aging were investigated for 12 months (Fig. 2). The total isoflavone content during fermentation/aging was 71.1–81.6 mg/100 g, showing that

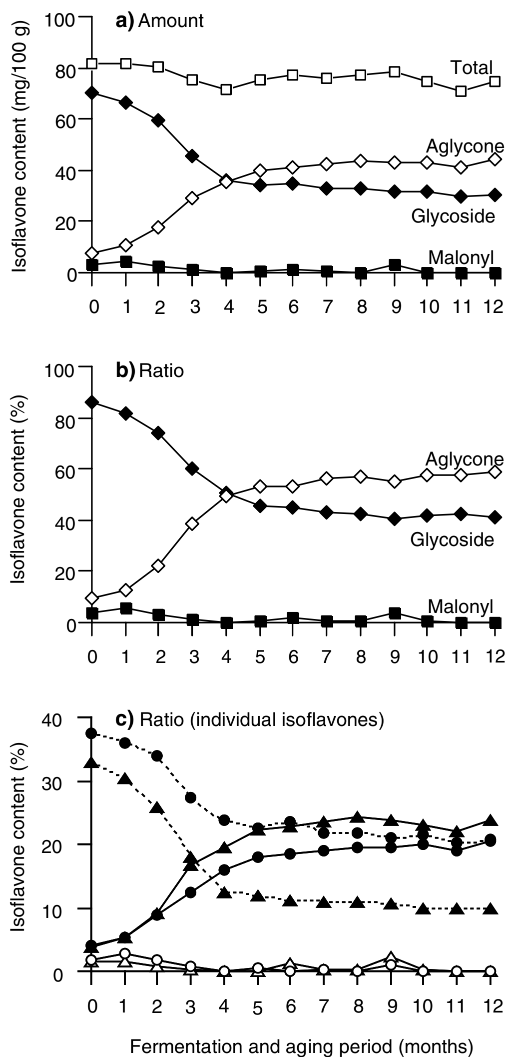

Fig. 2. Time course of isoflavone contents in rice-koji miso during fermentation and aging. rice-koji miso was prepared from soybean (Chinese soybeans) as described in Fig. 1. Isoflavone contents in rice-koji miso during fermentation and aging were determined by HPLC. Figure represents: (a) the amount of isoflavone types; (b) the ratio of isoflavone types to total isoflavone and (c) the ratio of each isoflavone to total isoflavone. Symbols in (c): malonyl daidzin (solid line with open triangle); malonyl genistin (solid line with open circle); daidzin (dashed line with closed triangle); genistin (dashed line with closed circle); daidzein (solid line with closed triangle) and genistein (solid line with closed circle).

fermentation/aging did not markedly change the total content (Fig. 2(a)). However, the contents of glycosides and aglycones significantly changed. Glycosides decreased from 70.5 to 34.6 mg/100 g by 6 months after initiation of brewing, while aglycones increased from 7.8 to 41.1 mg/100 g (Fig. 2(a)). Fig. 2(b) shows the ratios of malonyl glycosides, glycosides, and aglycones to the total isoflavone, and Fig. 2(c) shows the ratio of each isoflavone to the total isoflavone. The ratio of glycosides decreased from 86.4% to 44.9% by 6 months after initiation of the brewing process, while the ratio of aglycones increased from 9.6% to 53.3% (Fig. 2(b)). In particular, daidzin and daidzein markedly decreased and increased, respectively (Fig. 2(c)). These findings suggest that β-glycosidase was highly active from immediately after initiation of the brewing process (Murakami et al., 1984). The best time for eating rice-koji miso prepared by natural fermentation is about 6 months after initiation of the brewing process (Yamabe, Kaneko, Inoue, & Takita, 2004). The rice-koji miso prepared in this study contained 53.3% of aglycones at 6 months after initiation of the brewing process (Fig. 2(b)), showing that miso, ready to eat, contains abundant aglycones, which are more absorbable in the body (Izumi et al., 2000).

4. Conclusion

In recent health-consciousness trends, miso (a traditional Japanese fermented food), which has played an important role in Japanese diet, has become important as a source of isoflavone. In this study, we have shown that miso made from Tohoku-126 was superior in the quality and quantity of its isoflavone. Because the suitability of Tohoku-126 for processing has not been investigated, our work is valuable in that we revealed a high added value of miso made from Tohoku-126. Utilization of soybeans containing abundant isoflavone, such as Tohoku-126, for soybean products is expected.

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