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Influences of processing and NaCl supplementation on isoflavone contents and composition during douchi manufacturing

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Abstract

Douchi is popular in China as a fermented soybean food. The effects of NaCl on the isoflavone contents and composition during processing of douchi, as well as the β-glucosidase activity was investigated. The results indicate that 61% of the isoflavones in raw soybeans were lost when NaCl content was 10%, which is mainly attributed to pre-fermentation (43%) and post-fermentation (18%) during douchi processing. While a pre-treatment did not generate major differences in total isoflavone content during douchi processing, isoflavone composition was altered. The levels of aglycones increased, while the corresponding levels of β-glucosides, malonylglucoside and acetylglucoside decreased. Further, isoflavones in the form of aglycones exceeded 90% of total content following post-fermentation. Finally, changes in isoflavone isomer distribution were found to be related to β-glucosidase activity during fermentation, which was affected by NaCl supplementation.

Keywords: Douchi; Isoflavone; β-Glucosidase; NaCl

1. Introduction

Traditional fermented soybean-based foods have played an important role in human diets for centuries. There are many types of traditional fermented soybean foods in Asian countries such as miso, natto, tempeh, sufu and douche, among others. Douchi, which originated in China, has been consumed as a seasoning for food since ancient times. There are three types of douchi in China which are fermented by *Mucor*, *Bacteria* and *Aspergillus* strains, respectively. Among them, *Aspergillus*-type douchi is the most popular, with origins of production in China identifiable at least 2000 years ago (Bao, 1985).

Recently, an increasing number of people have become interested in the physiological properties of fermented soybean foods such as antioxidative activity, antiproliferative activity, and anti-hypertensive effects. The antioxidative and anti-hypertensive effect of sufu (Wang, Saito, Tatsumi, & Li, 2003; Wang, Li, Fan, Saito, & Tatsumi, 2004), the antioxidative activity of tempeh (Murakami, Asakawa, Terao, & Matsushita, 1984), and the antioxidative and antiproliferative activity of miso (Hirota, Taki, Kawaii, & Yano, 2000) have been well documented in the literature, where isoflavones have been found to be important compounds affecting soybeans.

The main isoflavones found in soybeans are daidzein, genistein and glycitein, each of which exists in four chemical forms: as an aglycones (daidzein, genistein and glycitein), a β-glucoside (daidzin, genistin and glycitin), an acetylglucoside (6″-*O*-acetyldaidzin, 6″-*O*-acetylgenistin

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and 6″-*O*-acetylglycitin), and a malonylglucoside (6″-*O*-malonyldaidzin, 6″-*O*-malonylgenistin and 6″-*O*-malonylglycitin) (Kudou et al., 1991). The content and composition of these isoflavones varies in soybean foods depending on processing techniques such as heat treatment, defoaming, enzyme hydrolysis, and fermentation (Anderson & Wolf, 1995; Jackson et al., 2002; Wang & Murphy, 1994a, 1994b, 1996). Several authors have reported that the isoflavone glucosides were hydrolyzed into their corresponding aglycones during the fermentation of soybean foods such as sufu (Yin, Li, Li, Tatsumi, & Saito, 2004), miso (Chiou & Cheng, 2001), natto (Ibe, Kumada, Yoshiba, & Onga, 2001) and tempeh (Murakami et al., 1984), while β-glucosidase has been considered to be a key enzyme for the conversion of isoflavone forms in sufu processing (Yin et al., 2004).

Isoflavone aglycones have shown different absorption patterns from that of glucosides in the stomach. For example, daidzein and genistein, unlike their glucoside form, were absorbed in rat stomachs (Piskula, Yamakoshi, & Iwai, 1999), while genistein was reported to have a higher antiproliferative effect on the growth of human breast carcinomas and prostate cancer cells than genistin (Onozawa et al., 1998; Peterson & Barnes, 1991). More recently, some of the *o*-dihydroxy structured isoflavones, 6-hydroxydaidzein (6-OHD), 8-hydroxydaidzein (8-OHD) and 8-hydroxyglycitein (8-OHG) have been found in various fermented soybean products (Esaki, Onozaki, Morimitsu, Kawakishi, & Osawa, 1998; Esaki, Kawakishi, Morimitsu, & Osawa, 1999). It is possible they were formed from daidzein or genistein by microbial hydroxylation during soybean fermentation/incubation with in the presence of *Aspergillus saitoi* (Esaki et al., 1998; Esaki, Watanabe, Onozaki, Kawakishi, & Osawa, 1999; Esaki, Kawakishi, et al., 1999; Hirota, Inaba, Chen, Abe, & Taki, 2004). These *o*-dihydroxy structured isoflavones were found to have a DPPH (α,α-diphenyl-β-pricrylhydrazyl) radical scavenging ability as high as that of α-tocopherol (Hirota et al., 2000). If so, then both the content and composition of isoflavones may influence the physiological functions in the processed food product.

Although douchi has been consumed in China for thousands of years, almost no information is available regarding changes in isoflavone content and composition during processing or the causes of these changes. In addition, NaCl is typically added to douchi to select microorganisms responsible for flavor and taste development and to inhibit the growth of pathogenic and spoilage microorganisms in order to extend shelf life. However, the degree to which NaCl content affects isoflavones in douchi is unknown. In the present study, we examined isoflavone content and composition by conducting mass balance studies during the preparation of douchi. We also investigated the relationship between the β-glucosidase activity and changes in isoflavone composition during processing and NaCl supplementation.

2. Materials and methods

2.1. Materials

Black soybeans, harvested in 2003, were provided by the Center of Soybean Research, Agricultural Academy of Jilin Province (Jilin, China). *Aspergillus oryzae* 3.951 was kindly donated by the Institute of Microbiology, Chinese Academy of Sciences (Beijing, China).

Daidzin, genistin, glycitin, daidzein, genistein, glycitein and *p*-nitrophenyl-β-D-glucoside (*p*-NPG) were purchased from Sigma Chemical Co. (St. Louis, MO). 6″-*O*-Malonyl-daidzin, 6″-*O*-malonyl-genistin, 6″-*O*-malonyl-glycintin, 6″-*O*-acetyl-daidzin, 6″-*O*-acetyl-genistin and 6″-*O*-acetyl-glycintin were purchased from Wako Pure Chemical Co., Ltd. (Osaka, Japan). All other chemicals were of reagent grade.

2.2. Douchi preparation

Douchi preparation was performed as described by Li, Li, and Yin (2003) as illustrated in Fig. 1. The steps and parameters were as follows:

(1) *Soybean pre-treatment*. Soybeans (1000 g) were washed then soaked in tap water (3300 ml) for 8 h at room temperature (24 ± 2 °C). After draining, the soybeans were steamed for 30 min at 121 °C in a retort (YMQ.L31.400, Beijing Jiangtai Medical Instrument Co., Ltd., Being, China).
(2) *Pre-fermentation*. The cooked soybeans were inoculated quickly with *Aspergillus oryzae* 3.951 after cooling to 30–35 °C. The rate of inoculation was 1×10^6 conidia per gram of cooked soybeans. Aliquots (200 g) of the soybeans mixed with fungi were placed in bamboo baskets (10.8 cm in diameter, 3.8 cm in depth) covered with wet cheesecloth and incubated at 30 °C for 60 h in an incubator with relative humidity maintained around 80% (LTI-601SD; Tokyo Rikakikai Co., Ltd, Tokyo, Japan). Semifinished products were called the douchi qu (Koji).

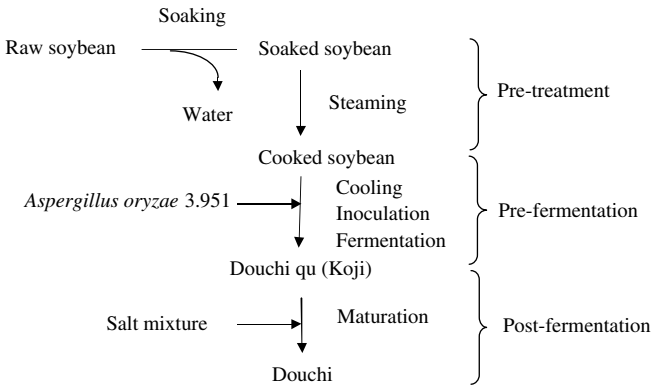

Fig. 1. Flow diagram for processing of soybeans to Douchi.

(3) *Post-fermentation*. Aliquots (200 g) of the douchi qu deposited in glass jars were salted until the NaCl content reached about 5%, 7.5%, 10% and 12.5% (w/w). The douchi obtained after each sample was ripened for 1 month at 35 °C.

Raw, soaked and cooked soybeans were sampled for analyses. Douchi qu samples were obtained after fermenting for 12, 24, 36, 48 and 60 h and douchi were sampled after ripening for 1, 2, 3 and 4 weeks, respectively.

The moisture contents of all samples were determined by freeze-drying to a constant weight to preserve isoflavones distribution. The moisture content data was then used to calculate isoflavone concentration in samples on a dry basis. The dried soybeans were pulverized into powder using a mortar and a pestle, and then used for isoflavone analysis.

2.3. Isoflavones extraction

Freezed-dried powder (2 g) was extracted with 50 ml of 80% methanol at 80 °C for 4 h by a Soxhlet extractor and the extractants filtered through a 0.45 μm filter unit.

2.4. High performance liquid chromatography (HPLC) analysis

Isoflavones were analyzed quantitatively by high pressure liquid chromatography (HPLC) with a Dikma Diamonsil C_{18} column (4.6 × 250 mm) (Dima Co., Ltd., Orlando, FL). The mobile phases for HPLC consisted of solvent (A) 0.1% (v/v) acetic acid in filtered MilliQ water, and (B) 0.1% (v/v) acetic acid in acetonitrile. The solvent gradient was as follows: Solvent B was increased from 15% to 25% over 35 min, then increased to 26.5% within the next 12 min, and finally increased to 50% within 30 s prior to being held for 14.5 min. The flow rate was 1.0 ml/min. The column temperature was 40 °C and the absorption was measured at 254 nm. Quantitative data for each isoflavone was obtained by comparison to known standards.

2.5. Determination of β-glucosidase activity

A modified Bahl and Agrawal (1968) procedure was used to determine β-glucosidase activity. Two grams of sample were homogenized with 20 ml of 0.2 M acetate buffer (pH 4.5) at 4 °C. The slurry was centrifuged at 10,000*g* for 10 min at 4 °C, and the supernatant used as a crude enzyme solution. Next, 2 ml of a 1 mM *p*-NPG solution and 0.5 ml of crude enzyme solution were mixed and incubated at 45 °C for 10 min. The reaction was stopped by addition of 2.5 ml of 1 M sodium carbonate. The resultant colour was immediately measured at 400 nm. One unit of enzyme activity was defined as the amount of enzyme that liberated 1 μM of *p*-nitrophenol per min.

2.6. Statistical analysis

All sample analyses were run in triplicate and statistical analysis done using SAS (version 6.12) developed by the SAS Institute Inc. (Cary, NC, USA). Duncan's multiple range tests were used to estimate significant differences among means at a probability level of 5%.

3. Results and discussion

3.1. Changes in the total contents of isoflavones during douchi processing

The yield, moisture, and isoflavone content results from douchi processing are presented in Table 1. The results show that 1000 g of soybean yielded 1546 g of douchi with 10% salt content. The procedure for producing douchi in this study resulted in a significant loss (61%) of total isoflavone content between the raw material and the douchi.

Pre-treatment did not generate major differences from raw soybeans in terms of total isoflavone content compared to fermentation. Our results suggest that fermentation leads to a significant loss of isoflavones with pre-fermentation contributing the principal source of the loss in producing douchi; generating the largest loss of isoflavones in douchi manufacturing. The total content of isoflavones in douchi qu was 365 mg compared to 684 mg in cooked soybeans with isoflavone losses from cooked soybean to douchi qu up to 43%. The results also show that the loss of isoflavones during post-fermentation was the second major cause of the decrease in total recovery of isoflavones. Eighteen percent of the total isoflavones were lost from douche qu. During this process, the losses of isoflavones were mainly resulted from the later stage of pre-fermentation and the former stage of post-fermentation. The total recovery of isoflavones decreased 28% in the course of pre-fermentation from 36 to 60 h, and 18% of total isoflavones were lost during post-fermentation after 1 week.

It has been suggested that some *o*-dihydroxy structure isoflavones are produced during fermentation with *Aspergillus saitoi*. Esaki, Watanabe, et al. (1999) reported that 8-OHD and 8-OHG were formed from daidzein and genistein, respectively, by microbial hydroxylation. However, these results assumed that the conversion of isoflavones associated with the hydroxylation derived from the microorganisms in fermented soybean food may be one reason for the losses of isoflavones during douchi fermentation. Our results suggest that it is possible that the aglycone isoflavones were converted to hydroxyl form under the microbial hydroxylation involved in douchi fermentation. Unfortunately, we did not analyze the contents of *o*-dihydroxy structured isoflavones and the properties of this fungi in the present study. Further research will be necessary to isolate and characterize the *o*-dihydroxy isoflavones from douchi.

Table 1
Yield, moisture, and isoflavone amounts[a,b] in douchi processing

Step	Yield (g)	Moisture (%)	Total daidzein (mg)	Total glycitein (mg)	Total genistein (mg)	Total (mg)
Raw soybeans	1000	9.46 ± 0.01	275.75 ± 29.52^{A}	17.78 ± 0.41^{A}	382.95 ± 32.92^{AB}	676.48 ± 62.03^{A}
Soaked soybeans	2026	54.25 ± 0.01	257.56 ± 17.99^{A}	17.13 ± 1.65^{A}	359.29 ± 23.58^{AB}	633.99 ± 39.92^{A}
Soaking water	NC[a]					
Cooked soybeans	1979	55.29 ± 0.45	276.11 ± 8.90^{A}	20.25 ± 0.98^{A}	387.70 ± 22.47^{A}	684.05 ± 32.36^{A}
12 h	1931	54.69 ± 0.19	261.01 ± 17.97^{A}	19.28 ± 0.64^{A}	373.99 ± 39.17^{AB}	654.280 ± 56.50^{A}
24 h	1900	55.03 ± 0.01	242.56 ± 29.31^{A}	17.66 ± 6.97^{A}	313.23 ± 15.88^{B}	573.45 ± 20.40^{A}
36 h	1726	49.42 ± 0.04	234.16 ± 3.03^{AB}	15.59 ± 3.10^{A}	306.98 ± 5.28^{B}	556.73 ± 0.85^{A}
48 h	1674	49.55 ± 0.01	232.84 ± 18.76^{ABC}	11.01 ± 1.04^{B}	225.85 ± 40.75^{C}	469.69 ± 60.56^{B}
60 h	1616	48.44 ± 0.30	193.13 ± 10.99^{BCD}	7.82 ± 0.55^{B}	164.27 ± 27.10^{C}	365.22 ± 38.64^{B}
1wN10[c]	1650	44.52 ± 0.01	171.02 ± 38.02^{D}	6.89 ± 1.19^{B}	60.56 ± 9.17^{D}	238.48 ± 48.38^{C}
2wN10[c]	1654	44.77 ± 0.01	178.68 ± 33.12^{D}	7.82 ± 1.89^{B}	62.92 ± 13.12^{D}	249.42 ± 58.13^{C}
3wN10[c]	1595	42.95 ± 0.01	190.52 ± 25.71^{D}	8.90 ± 2.17^{B}	55.48 ± 22.41^{D}	254.90 ± 50.29^{C}
4wN10[c]	1546	41.26 ± 0.01	198.01 ± 28.24^{BD}	8.90 ± 1.17^{B}	57.38 ± 10.02^{D}	264.29 ± 43.64^{C}

[a] In order to estimate total isoflavone amounts, individual isoflavone glucodides and aglycones were normalized for their molecular weight differences and summed. Values represent the means ± SD; $n = 3$. Values in a column with different superscripts were significantly different ($p < 0.05$). NC, not collected.
[b] Calculated on dry basis.
[c] N10 = 10% NaCl content in douchi. Yield was estimated by dry basis.

No significant changes in the total content of isoflavones were observed under various salt content schemes in douchi (data no shown).

3.2. Changes in the mass distribution profile of isoflavone isomers during douchi processing

The mass distribution profiles of individual isoflavone isomers are shown in Table 2. During the entire processing procedure from raw soybeans to douchi, the aglycones (daidzein, genistein and glycitein) increased, while the β-glucosides (daidzin, genistin and glycitin), malonylglucosides (6″-*O*-malonyldaidzin, 6″-*I*-malonylgenistin and 6″-*O*-malonylglycitin), and acetylglucosides (6″-*O*-acetyldaidzin and 6″-*O*-acetylgenistin) decreased. Further, both raw and soaked soybeans exhibited comparative isoflavone profiles with the soaked soybeans showing an increase in the amount of isoflavone aglycones accompanied by a decrease in the amount of isoflavone glucosides (β-glucosides, malonylglucosides and acetylglucosides) in comparison with the raw soybeans. Cooking of soybean decreased malonylglucosides (6″-*O*-malonyldaidzin, and 6″-*O*-malonylgenistin) and increased the amounts of β-glucosides and acetylglucoside forms, but did not cause considerable alteration of isoflavone distribution. Table 2 also shows that during the fermentation, which included pre-fermentation and post-fermentation, fermentation could cause a significant increase in the amount of isoflavone aglycones accompanied by a decrease in isoflavone glucosides. As a consequence, most of the isoflavones in douchi occurred in the form of aglycones. 6″-*O*-Acetylglycitin was not detected in any of the samples.

This work suggests that the processing steps, soaking, cooking and fermentation, examined in this study could

Table 2
Effects of douchi processing on the mass distribution profile of isoflavone isomers (mg)[a,b]

Step	β-Glucoside[c]			Aglycone[c]			Malonyl glucoside[c]			Acetyl glucoside[c]	
	Din	Glin	Gin	Dein	Glein	Gein	Din	Glin	Gin	Din	Gin
Raw soybeans	324.94^{ABC}	27.93^{A}	391.08^{B}	3.24^{D}	ND	1.49^{F}	131.77^{A}	ND	234.01^{A}	13.14^{ABC}	26.65^{AB}
Soaked soybeans	288.04^{ABC}	25.20^{A}	369.07^{B}	22.00^{C}	1.09^{D}	7.55^{EF}	107.31^{A}	ND	211.25^{B}	9.49^{ABC}	19.41^{ABC}
Cooked soybeans	358.40^{A}	29.39^{A}	448.20^{A}	7.67^{C}	ND	4.79^{EF}	75.61^{B}	2.88^{A}	149.82^{BC}	20.16^{AB}	43.50^{A}
12 h	333.97^{AB}	27.04^{A}	428.97^{AB}	12.29^{C}	0.43^{D}	5.65^{EF}	64.22^{BC}	3.05^{A}	136.34^{BC}	21.90^{AB}	51.35^{A}
24 h	240.84^{ABC}	22.87^{A}	320.20^{B}	50.01^{B}	1.62^{DC}	15.73^{DC}	65.43^{BC}	2.77^{A}	139.03^{BC}	22.14^{A}	43.86^{A}
36 h	252.36^{ABC}	21.09^{A}	331.87^{B}	39.64^{BC}	1.02^{D}	12.76^{DE}	60.03^{C}	2.15^{A}	127.22^{C}	17.91^{AB}	36.09^{A}
48 h	105.33^{BCD}	8.43^{B}	184.76^{C}	145.29^{A}	5.64^{ABC}	48.60^{A}	27.68^{D}	ND	87.32^{D}	16.55^{AB}	28.64^{AB}
60 h	78.71^{DC}	5.12^{BC}	143.48^{C}	129.31^{A}	4.57^{BC}	36.01^{B}	19.01^{DE}	ND	55.42^{D}	11.00^{ABC}	17.08^{ABC}
1wN10[d]	19.85^{D}	0.80^{C}	51.75^{D}	156.72^{A}	6.38^{ABC}	21.83^{C}	ND	ND	7.27^{E}	3.92^{C}	4.57^{C}
2wN10[d]	9.70^{D}	0.52^{C}	28.08^{D}	166.92^{A}	7.48^{AB}	42.04^{A}	5.99^{DE}	ND	6.38^{E}	5.06^{C}	ND
3wN10[d]	4.35^{D}	0.43^{C}	18.47^{D}	182.45^{A}	8.62^{A}	41.77^{A}	4.45^{DE}	ND	4.16^{E}	5.70^{C}	ND
4wN10[d]	2.24^{D}	0.52^{C}	10.30^{D}	191.63^{A}	8.57^{A}	50.94^{A}	2.49^{DE}	ND	ND	6.75^{C}	ND

[a] Values represent the mean; $n = 3$.Values in a column with different superscripts were significantly different ($p < 0.05$).
[b] Calculated on dry basis as milligrams of individual isomer.
[c] Din, daidzin; Gin, genistin; Glin, glycitin; Dein, daidzein; Gein, genistein; Glein, glycitein. ND, not detected.
[d] N10 = 10% NaCl content in douchi.

cause significant differences in the redistribution of isoflavone isomers, and which have previously been documented (Kudou et al., 1991; Matsuura, Obata, & Fukushima, 1989; Wang & Murphy, 1996; Yin et al., 2004). Matsuura et al. (1989) confirmed that the endogenous β-glucosidase enzyme present in soybeans was able to convert isoflavone glucosides into aglycones, and thus lead to an increase in the amount of isoflavone aglycones during the soaking stage. This was accompanied by a decrease in the amount of isoflavone glucosides. Interestingly, this conversion associated with endogenous β-glucosidase in soybeans has only observed prior to heat treatment (Ha, Morr, & Seo, 1992). Kudou et al. (1991) reported that the acetyl derivatives might have arisen from corresponding malonyl derivatives during heat treatment. Fermentation may also generate a more significant change in isoflavone distribution than soaking or heat treatment (Wang & Murphy, 1996; Yin et al., 2004). Here, the aglycone content increased from 1.82% to 95.02% between cooked soybeans and douchi (10% salt content) suggesting that the douchi was rich in isoflavone aglycones and that douchi manufacturing might be beneficial to the enhancement of the physiological function.

3.3. Changes in the β-glucosidase activity and percentage of isoflavone aglycones during douchi processing at different salt content

The change in β-glucosidase activity during the pre-treatment stage of douchi processing is presented in Table 3, where the β-glucosidase activity in both raw and soaked soybeans was higher than that in the cooked soybean. We also detected a low level of thee β-glucosidase activity in heated soybeans suggesting that most of the endogenous enzymes were presumably inactivated by the heat treatment.

The changes in β-glucosidase activity and the percentage of isoflavone aglycones produced during douchi pre-fermentation are shown in Fig. 2. Both β-glucosidase activity and the percentage of isoflavone aglycones increased significantly during the pre-fermentation ($p < 0.05$). The change in the percentage of isoflavone aglycones was significantly related to the activity of β-glucosidase ($r = 0.999$).

Fig. 3 shows the changes in β-glucosidase activity during douchi post-fermentation. When the salt content of douchi was 10%, the β-glucosidase activity increased from 325.56 U/g dry matter in post-fermentation 1 week to 367.78 U/g dry matter in 4 week, with the highest level of β-glucosidase activity (403.89 U/g dry matter) recorded during the third week of the post-fermentation procedure. The level of the β-glucosidase activity decreased with the increase of NaCl content at the same fermentation time ($p < 0.05$). β-Glucosidase activity could remain high if the NaCl content was 5%, although β-glucosidase activity was considerably inhibited by higher NaCl contents (7.5%, 10% and 12.5%). β-glucosidase activity was almost 1.32 times higher at 5% NaCl content than at 12.5% after the post-fermentation stage.

Fig. 4 shows the percentage of isoflavone aglycones during douchi post-fermentation, suggesting that the percentage of isoflavone aglycones in douchi increased significantly during the first 3 weeks of post-fermentation ($p < 0.05$). The level of NaCl content did affect the percentage of isoflavone aglycones, which decreased with increases in NaCl content. Isoflavone aglycones percentages were found to be 96.71% at 5% NaCl content and 90.00% at

Table 3
Changes in the β-glucosidase activity for pre-treatment of douchi processing

Step	Activity (U/g dry matter)[a]
Raw soybeans	83.89 ± 3.14^{B}
Soaked soybeans	90.00 ± 3.93^{A}
Cooked soybeans	22.22 ± 2.36^{C}

[a] Values represent the mean ± SD; $n = 3$. Values in a column with different superscripts were significantly different ($p < 0.05$).

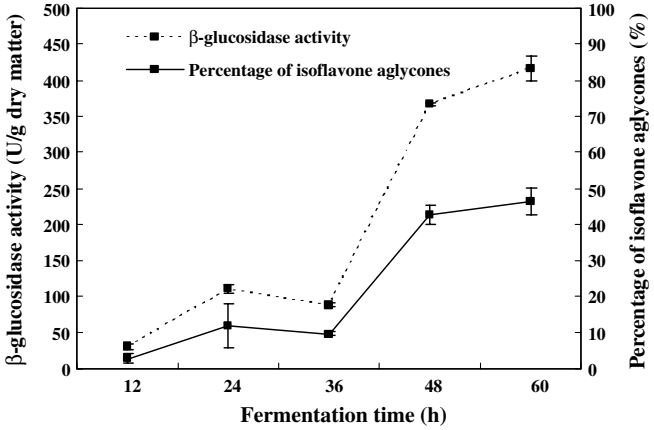

Fig. 2. Changes in the β-glucosidase activity and percentage of isoflavone aglycones during douchi pre-fermentation. Results are means ± SD of three determinations.

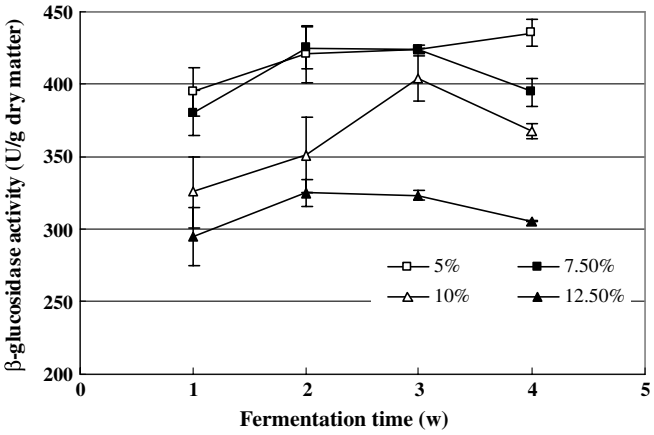

Fig. 3. Changes in the β-glucosidase activity during douchi post-fermentation at various NaCl content. Results are means ±SD of three determinations.

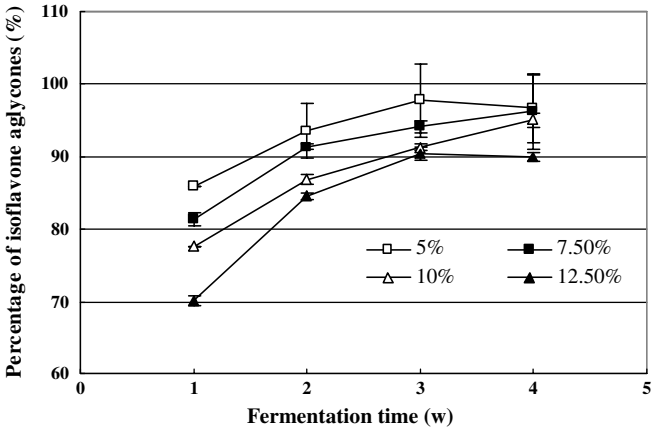

Fig. 4. Changes in the percentage of isoflavone aglycones during douchi post-fermentation at various NaCl content. Results are means ± SD of three determinations.

12.5% NaCl content. After post-fermentation, the percentage of isoflavone aglycones exceeded 90% in all samples.

In this study, the increase in the percentage of isoflavone aglycones kept a relatively close correspondence with the changes in β-glucosidase activity during fermentation. The release of aglycones from glucosides appeared to be due to the hydrolysis by β-glucosidase during douchi fermentation. It was assumed that the enzyme hydrolyzing isoflavones from glucosides to aglycones was derived from microorganisms involved in douchi fermentation since most of the endogenous enzymes were presumably inactivated by the heat treatment in cooked soybeans (Table 3).

As mould starters, a pure culture of *Aspergillus oryzae* 3.951 was used in douchi processing. It is possible that the fungi produced β-glucosidase and hydrolyzed isoflavone glucosides to aglycones, although the properties of this fungi were not analyzed in the present study. It is possible that the fungi, *Aspergillus oryzae* 3.951 used here was not metabolically active under a high NaCl content, or that the high NaCl content inhibited the β-glucosidase activity generated by the fungi. If so, then this may have prevented the conversion of isoflavone glucosides into aglycones.

Findings of this study suggest that the low NaCl content douchi was richer in isoflavone aglycones than douchi with a high NaCl content. Several reports have mentioned that isoflavones, in the form of aglycones, display a higher bioavailability than isoflavones in the form of glucosides (Onozawa et al., 1998; Peterson & Barnes, 1991; Piskula et al., 1999). As such, douchi with a low NaCl content may be preferable from the viewpoint of public nutritional health, especially at the present, and that a high salt level in traditional foods is a serious concern for consumers due mainly to health considerations.

4. Conclusions

The results of the present work indicate douchi, a traditional fermented soybean food, possessing high content of isoflavone aglycones. Precessing step in making douchi and NaCl content affected the isoflavone contents and composition, and the activity of β-glucosidase in douchi. Pre-fermentation generated the largest losses of isoflavones in douchi manufacturing. During douchi making, the content of isoflavone aglycones increased, while isoflavone glucosides decreased, due to the hydrolysis by β-glucosidase. A high NaCl content in douchi inhibited the β-glucosidase activity and resulted in preventing isoflavone glucosides from being converted into aglycones. Furthermore, high NaCl content of food could increase the dietary sodium intake (Baggott et al., 1990). Therefore, douchi with a low NaCl content may be preferable from the viewpoint of public nutritional health.

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