Received: 24 September 2009 Revised: 19 November 2009 Accepted: 21 December 2009 Published online in Wiley Interscience: 15 February 2010

(www.interscience.wiley.com) DOI 10.1002/jsfa.3894

Effects of the β-glycosidase reaction on bio-conversion of isoflavones and quality during tofu processing

Young-Gi Ryu,[a] Bomi Won,[b] Hae-Ryung Park,[b] Kashif Ghafoor[b] and Jiyong Park[b]*

Abstract

BACKGROUND: Isoflavones are the most common group of phytoestrogens which are present in significantly large amounts in soybean and soy products such as tofu. Isoflavones occur naturally in glycoside forms having lower bioavailability than their aglycone forms. β-Glycosidase acts as a bio-catalyst for the conversion of isoflavone glycosides to isoflavone aglycones, raising the bioavailability of isoflavones; therefore, it can be used to improve the quality of tofu. We need to establish process conditions for the optimal outcome of the enzyme reaction in tofu.

RESULTS: By using the β-glycosidase (0.02% w/v) reaction at 55 °C for 30 min, a maximum 84.5% conversion of isoflavone glycoside to isoflavone aglycone was obtained. The enzyme reaction caused no significant effects on the sensory acceptability of soft tofu. The hardness of enzyme-treated hard tofu increased with the coagulant amount whereas prolonged heating resulted in decrease of hardness. Incorporation of enzyme reaction before the coagulation process during soft tofu processing provided a sufficient bio-conversion of isoflavones at optimal conditions.

CONCLUSION: β-Glycosidase can be effectively used for the bioconversion of isoflavones in soft tofu manufacturing process at optimal reaction conditions before the onset of coagulation process.

Keywords: tofu processing; β-glycosidase; isoflavones; bioconversion; tofu quality

INTRODUCTION

Soybean (*Glycine max*) is one of the largest agricultural products having high commercial value due to its good nutritional content. It is easy to cultivate, possesses immunity to germs and insects, and is convenient for processing and cooking.[1] Soybeans contain sufficient amounts of isoflavones, a group of plant oestrogens which have structural and functional similarities to human oestrogens.[2,3] Due to these similarities to the human oestrogens, isolated isoflavones are reported to retard bone loss.[4,5] Higher isoflavone intake is associated with better vascular endothelial function in patients with a high risk of cardiovascular disease.[6,7] These are also promising candidates for cancer prevention, including breast and prostate cancer.[3,8] Soybean isoflavones are generally present in the glycoside form, which has a sugar component bound to a hydroxyl group of the aglycone.[9,10] The isoflavone glycoside is poorly absorbed in the small intestine and is less bioactive than the aglycone form due to its higher molecular weight and hydrophilicity.[10–13] The conversion of the isoflavone glycosides to isoflavone aglycones in soy products can increase the bioavailability and hence improve the isoflavone associated health benefits. Tofu is a popular product of soybean made by coagulation of soymilk to form a protein matrix. It is a widely consumed food in countries of the Far East, such as China, Japan and Korea. It is also being considered as a meat alternative in Western countries, such as USA, UK and France.[14] It is a low-calorie food due to the presence of significantly higher amounts of water and contains essential amino acids, fatty acids, calcium, and several useful phytochemicals originating from soybeans.[15] Among all the naturally occurring phytochemicals in tofu, isoflavones are present in considerably large amounts. The β-glycosidase is an enzyme capable of catalysing the bio-conversion of isoflavone glycoside to isoflavone aglycone containing a non-sugar component, aglycone.[16,17] Therefore this enzyme can be used during tofu processing for improving the bioavailability of isoflavones. Most of the research on isoflavones has focused on increasing their intake from tofu and other soy products. However, less attention has been paid on the conversion of isoflavone glycoside, which has higher stability and lower absorption in the intestines, to a readily available form. Higher contents of isoflavone aglycone in enzyme-treated tofu can benefit consumers due to the increased health benefits of isoflavones as compared to the conventionally processed one.

* Correspondence to: Jiyong Park, Biomaterial Process Engineering Laboratory, Department of Biotechnology, Yonsei University, 134-Sinchon Dong, Seodaemun-Gu, Seoul, 120-749, South Korea. E-mail: foodpro@yonsei.ac.kr

a R&D Center, Pulmuone Co., Seoul 120-600, Korea

b Department of Biotechnology, Yonsei University, Seoul 120-749, Korea

The purpose of our study was to develop a tofu that is rich in isoflavone aglycone by using β-glycosidase. We also aimed at predicting the optimum process conditions for the enzyme reaction and its effect on the quality of soft and firm tofu.

MATERIALS AND METHODS

Materials

The domestic soybean cultivar 'Danbaek' from Yoojin Nursery Company (Daejeon, Chungnam, Korea), harvested in spring, was used in this study. β-Glycosidase derived from *Aspergillus fumigatus* was purchased from Amano Enzyme Inc. (Nagoya, Owari, Japan). Soy-Mineral® (emulsified magnesium chloride), and Soy-Mineral 5® (a mixture of glucono-δ-lactone, calcium sulfate, citric acid and magnesium chloride) were used as coagulants and purchased from Taejin G & S (Incheon, Gyeonggi, Korea). Methanol, ethanol, butylated hydroxytoluene (BHA), trifluoroacetic acid (TFA), glycitein, genistein and daidzein were of HPLC grade and purchased from Sigma–Aldrich (St Louis, MO, USA). Water was purified using a Milli-Q water purifier system (Millipore, Bedford, MA, USA).

Preparation of soymilk

One kilogram of soybeans was soaked in water at 20 °C for 12 h and then ground in a wet grinding mill (D235, Sungchang Co., Seoul, Korea) using a water flow of 4.5 L min^{-1}. The ground soybean mixture was steamed at 196 kPa pressure for 3 min, centrifuged at 6900 × *g* rpm for 5 min and finally passed through a filter cloth to separate the insoluble residue called 'okara' from the filtrate. Soymilk was collected and maintained at 10 °C for further experiments.

Production of tofu

Firm and soft types of tofu were prepared following two different processing procedures. The procedure for firm tofu incorporates pressing after filtration. Soft tofu has a fine texture with 88–90% water content and it is packed in containers together with water.[18] In order to make firm tofu soymilk was heated to 85 °C and mixed with Soy-Mineral® 0.8% (w/v). The coagulant–soymilk mixture was maintained at 85 °C for 20 min to obtain soybean curd, which was then poured onto tofu trays and pressed for 20 min using a weight of 20 kg. The pressed firm tofu was placed into a water-filled polypropylene box, sealed, heated at 85 °C in a water bath (NTS 3100; Tokyo Rikakikai Co. Ltd., Tokyo, Japan) for 40 min and finally cooled to 5 °C. In order to make soft tofu, soymilk was cooled to 10 °C and Soy-Mineral 5® 0.3% (w/v) was mixed. The coagulant–soymilk mixture was put into a polypropylene box and sealed. The sealed box was heated at 85 °C in a water bath for 30 min and coagulation was induced during heating.

Determination of the effects of process variables on isoflavones during the β-glycosidase reaction

In order to determine the optimum reaction temperature, the coagulant Soy-Mineral 5® 0.3% (w/v) and β-glycosidase 0.10% (w/v) were mixed with soymilk. The mixture was poured into a polypropylene box and sealed. The sealed soymilk mixture was placed in 25, 50 and 70 °C water bath for 60 min to obtain a soybean curd. Soybean curd was then heated at 85 °C for 40 min to form the tofu which was freeze-dried before analysis for isoflavone contents. The total isoflavones were determined by summation of the quantities of isoflavone aglycones and isoflavone glycosides. The conversion rates of isoflavone glycosides into aglycone types using the enzyme treatment were calculated by dividing the total isoflavone aglycones by the total isoflavones amount.

To determine the optimum reaction time of β-glycosidase, Soy-Mineral 5® 0.3% (w/v) and β-glycosidase 0.06% (w/v) were mixed with soymilk. The mixture was put in a polypropylene box and sealed. The sealed soymilk mixture was placed in a water bath at 55 °C for 10, 30 and 60 min to obtain a soybean curd. Other procedures followed were same as for the optimisation of temperature. Similarly for the determination of optimum β-glycosidase concentration, Soy-Mineral 5® 0.3% (w/v) and β-glycosidase 0.01–0.10% (w/v) were mixed with soymilk. The mixture was sealed in a polypropylene box and incubated at 55 °C for 30 min to obtain a soybean curd. The next steps were same as for the determination of optimum temperature and time.

Production of β-glycosidase-treated soft tofu

β-Glycosidase-treated soft tofu was produced following two different processing methods. In Process 1, it was intended to induce the β-glycosidase reaction before the coagulation process, whereas in Process 2, the β-glycosidase reaction was induced during the coagulation process as presented in Fig. 1. β-Glycosidase (0.02% w/v) reactions for both processes were performed at 55 °C for 30 min.

Analysis of isoflavones

Isoflavones from β-glycosidase-treated tofu were extracted according to previously reported procedures[19,20] with little modification. For the analysis of isoflavone aglycone, each sample was extracted using 2 mol L^{-1} HCl in an ethanol–water solution (50 : 50, v/v) containing 0.05% BHT for 4 h at 85 °C. Analysis of isoflavone glycoside was carried out by the extraction of sample using 100% ethanol. Following the extraction, samples were sonicated for 5 min; the insoluble residue was separated using centrifugation and the supernatant filtered for HPLC analysis using a 0.45 µm syringe filter (Millipore Co., Bedford, MA, USA). The high-performance liquid chromatography (HPLC) system was from Dionex Corporation, Sunnyvale, CA, USA consisting of an HPLC pump (P-680), automated sample injector (ASI-100) and thermostat column compartment (TCC-100). A Gemini (Phenomenex, Torrance, CA, USA) 5U C18 110A, 250 × 4.6 mm, 5 µm column was used. The mobile phase, composed of 0.1% TFA in distilled water (solvent A) and 0.1% TFA in methanol (solvent B), was used at a flow rate 1 mL min^{-1}. Solvent A was decreased from 60 to 40% over 45 min, 60 to 20% over 5 min and then held at 20% for 10 min. The injection volume was 10 µL and eluted isoflavones were detected at 254 nm.

Hardness and sensory properties of tofu

The hardness of firm tofu was determined using a rheometer (CR 500DX; Sun Scientific Co., Tokyo, Japan). Tofu was cut into 3 cm cubes from the central portion using a cylindrical plunger with 2.5 cm diameter and a weight beam of 2 kg. The cross-head was set at 60 mm min^{-1}, table speed had clearance of 5 mm, and the plunger travelled 40% of the depth into the tofu cube. The sensory analysis of tofu was carried out at Dong-Seo Research Co. (Seoul, Korea). The sensory acceptability attributes for colour, surface appearance, taste, texture, and overall acceptability were tested using a five-point scale (5 being very good, and 1 being very bad). Samples were evaluated by 150 female panelists aged between 30 and 60 years.

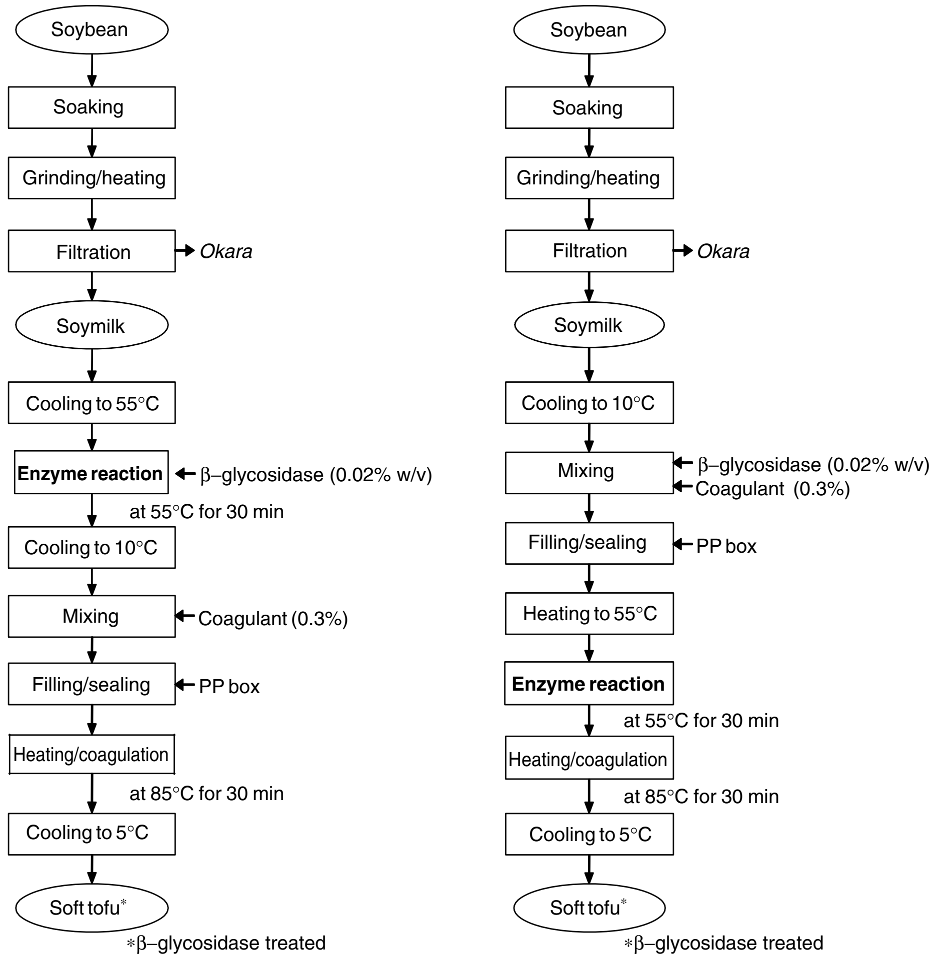

Figure 1. Different processing methods, Process 1 (left) and Process 2 (right) for β-glycosidase-treated soft tofu.

Statistical analysis

Analytical values were expressed as mean ± standard deviation. Differences in the mean scores of sensory properties of enzyme treated tofu and control were analysed by using analysis of variance (ANOVA) and Duncan's range test using SPSS software (SPSS Inc., Chicago, IL, USA). Significance was defined at $P < 0.05$.

RESULTS AND DISCUSSION

Effects of process variables on the β-glycosidase reaction of isoflavones in soft tofu

The belief that the aglycone is more bioavailable than its respective glycoside has led to the development of aglycone-enriched products such as by the application of β-glycosidase.[21] Different studies have also been carried out to determine the optimal processing conditions for maximum isoflavone activities in tofu.[22] In our study we found that during the process for making soybean curd, β-glycosidase-mediated conversion rates of isoflavone glycoside to isoflavone aglycone in soft tofu were 83.6% and 84.6% at 50 and 70 °C, respectively (Table 1). The reaction rate at these temperatures was significantly higher than at 25 °C. However, it was not markedly influenced by increasing the temperature from 50 to 70 °C. These reactions were carried out by the application of heat for 60 min.

It has been reported that prolonged heating at higher temperatures may lower the nutritional quality of tofu.[23] Therefore, to determine a suitable time duration for the application of heat, reactions were carried out for different times at a constant temperature of 55 °C. The data showing the effects of time on the bio-conversion of isoflavones during soybean curd making is presented in Table 2. It can be observed that only 55% of isoflavone glycosides were converted to isoflavone aglycones after 10 min of enzyme reaction; however, the conversion rate increased to 85.8% after 30 min reaction time. There were no significant changes in the conversion rates by raising the reaction time beyond 30 min; therefore the β-glycosidase reaction time of 30 min at 55 °C was used for further experiments.

As reported earlier, while studying the activity of β-glycosidase towards isoflavones it was noted that the reaction is significantly affected by the amount of enzyme used.[21] β-Glycosidase concentrations in the range of 0.01–0.10% (w/v) were used in our study to find an optimal amount for the reaction. The conversion rate increased up to 84.5% at 0.02% enzyme concentration; beyond this concentration there was no significant increase in the conversion rate (Table 3).

While optimising the reaction parameters for enzyme activity we also studied the effects of enzyme on three kinds of isoflavones in soft tofu, namely daidzein, glycitein and genistein. The data (Tables 1–3) reveal that the glycoside form of glycitein in tofu showed the lowest conversion rate to its aglycone form at different conditions of temperature, time and enzyme concentration. Apparently, the β-glycosidase had a higher affinity toward daidzein

Table 1. Effects of the β-glycosidase reaction temperature on the conversion of glycoside isoflavone to aglycone isoflavone in soft tofu

		Isoflavone content (mg 100 g^{-1}, dry basis)[a]			
Reaction temperature (°C)	Type	Daidzein	Glycitein	Genistein	Total
25	Isoflavone aglycone	8.42 ± 0.67	1.58 ± 0.13	9.78 ± 0.78	19.78 ± 1.57
	Total isoflavone	73.97 ± 0.79	31.47 ± 0.34	77.19 ± 0.82	182.63 ± 1.95
	Conversion rate (%)	–	–	–	10.8
50	Isoflavone aglycone	53.83 ± 7.58	19.26 ± 5.91	74.21 ± 2.52	147.31 ± 3.25
	Total isoflavone	72.03 ± 0.86	30.44 ± 0.37	73.64 ± 1.37	176.11 ± 2.32
	Conversion rate (%)	–	–	–	83.6
70	Isoflavone aglycone	53.81 ± 8.08	19.19 ± 5.72	74.05 ± 1.00	147.06 ± 3.46
	Total isoflavone	71.06 ± 0.80	30.03 ± 0.45	72.65 ± 1.85	173.74 ± 3.05
	Conversion rate (%)	–	–	–	84.6

[a] Means ± SD ($n = 3$).
The reaction time was 60 min and the β-glycosidase concentration was 0.10%.

Table 2. Effects of the β-glycosidase reaction time on the conversion of glycoside isoflavone to aglycone isoflavone in soft tofu

		Isoflavone contents (mg 100 g^{-1}, dry basis)[a]			
Enzyme reaction time (min)	Type	Daidzein	Glycitein	Genistein	Total
0	Isoflavone aglycone	8.42 ± 0.67	1.58 ± 0.13	9.78 ± 0.78	19.78 ± 1.57
	Total isoflavone	73.97 ± 0.79	31.47 ± 0.34	77.19 ± 0.82	182.63 ± 1.95
	Conversion rate (%)	–	–	–	10.8
10	Isoflavone aglycone	30.96 ± 1.25	16.14 ± 0.65	50.70 ± 2.05	97.79 ± 3.96
	Total isoflavone	71.99 ± 2.48	30.61 ± 1.05	74.83 ± 2.58	177.43 ± 6.11
	Conversion rate (%)	–	–	–	55.1
30	Isoflavone aglycone	55.50 ± 1.62	23.34 ± 0.68	78.62 ± 2.30	157.46 ± 4.61
	Total isoflavone	75.04 ± 1.29	31.58 ± 0.54	76.88 ± 1.32	183.50 ± 3.15
	Conversion rate (%)	–	–	–	85.8
60	Isoflavone aglycone	51.77 ± 1.79	22.77 ± 0.75	76.66 ± 2.51	154.19 ± 5.05
	Total isoflavone	75.41 ± 2.19	31.78 ± 0.92	76.07 ± 2.20	183.27 ± 5.31
	Conversion rate (%)	–	–	–	84.1

[a] Means ± SD ($n = 3$).
The reaction temperature was 55 °C and the β-glycosidase concentration was 0.06%.

and genistein than glycitein and the results are in accord with those reported by Ismail and Hayes.[21]

Effects of coagulant and β-glycosidase treatment on the hardness of firm tofu

The hardness is one of the several parameters that ultimately determine the quality of tofu, e.g. a firm tofu should be one being harder and firmer. The coagulation of soymilk is an important step for determining the hardness of tofu and the amount of coagulant used also plays a significant role.[23] In our study the changes in the hardness of firm tofu during β-glycosidase treatment using different coagulant concentrations (0.2, 0.3 and 0.5%) were measured at pre-determined enzyme reaction conditions (0.02% β-glycosidase concentration and 55 °C temperature) for 0–60 min reaction time. We can observe in Fig. 2 that the hardness of tofu increased with the increase in concentration of coagulant. The hardness of the firm tofu did not change significantly until 30 min of enzyme reaction, and afterwards it started to decrease regardless of the coagulant concentration. The decrease in hardness was apparently caused by longer enzyme reaction time which had negative affects on the network structure of tofu.[24] It has been reported that the coagulant concentration and the type of anions forming network structures might also affect the hardness of tofu.[25] Similarly, Saio[26] also reported that the density of the network of tofu correlates with the hardness of tofu; however, it was pointed out by Noh *et al.*[23] that without heat treatment, soymilk, even in the presence of coagulant, only produced a paste-like gel, which implies the significance of optimal temperature for the desired hardness in tofu. We may infer that besides proper heating during tofu processing, optimal conditions for the enzyme reaction and the proper concentration of coagulant are important for obtaining the desired hardness in enzyme-treated firm tofu and for preserving its unique texture.

Sensory properties of β-glycosidase-treated soft tofu

The data of sensory analysis of β-glycosidase-treated soft tofu prepared at pre-determined reaction conditions (β-glycosidase concentration 0.02%, temperature 55 °C, and reaction time 0–60 min) revealed that the enzyme-treated soft tofu showed non-significant ($P > 0.05$) differences in the sensory scores for different attributes as compared to the control, tofu prepared without enzyme treatment. The sensory attributes analysed were

Table 3. Effects of the β-glycosidase reaction concentration on the isoflavone contents in soft tofu

Enzyme concentration (%)	Type	Isoflavone content (mg 100 g^{-1}, dry basis)[a]			
		Daidzein	Glycitein	Genistein	Total
0	Isoflavone aglycone	7.23 ± 2.48	3.04 ± 1.05	10.25 ± 3.52	20.52 ± 7.05
	Total isoflavone	55.85 ± 9.05	15.53 ± 7.07	72.12 ± 2.18	143.50 ± 1.84
	Conversion rate (%)	–	–	–	14.3
0.01	Isoflavone aglycone	11.46 ± 1.44	5.97 ± 0.75	18.77 ± 2.36	36.21 ± 4.56
	Total isoflavone	60.42 ± 2.10	15.87 ± 7.52	67.26 ± 7.44	143.55 ± 2.80
	Conversion rate (%)	–	–	–	25.2
0.02	Isoflavone aglycone	49.17 ± 9.81	13.47 ± 5.60	63.20 ± 2.92	125.84 ± 7.32
	Total isoflavone	61.26 ± 3.17	25.82 ± 1.33	61.79 ± 3.19	148.87 ± 7.69
	Conversion rate (%)	–	–	–	84.5
0.04	Isoflavone aglycone	50.92 ± 1.88	13.45 ± 6.61	56.63 ± 5.73	121.00 ± 4.02
	Total isoflavone	58.61 ± 1.37	24.67 ± 0.58	60.05 ± 1.40	143.33 ± 3.35
	Conversion rate (%)	–	–	–	84.4
0.06	Isoflavone aglycone	50.37 ± 0.94	21.23 ± 0.40	50.81 ± 0.95	122.41 ± 2.29
	Total isoflavone	59.78 ± 0.73	25.33 ± 0.43	61.84 ± 1.18	146.95 ± 2.33
	Conversion rate (%)	–	–	–	83.3
0.08	Isoflavone aglycone	51.93 ± 2.02	21.85 ± 0.85	53.20 ± 2.07	126.98 ± 4.94
	Total isoflavone	59.53 ± 2.11	25.32 ± 0.89	61.96 ± 2.07	146.81 ± 5.07
	Conversion rate (%)	–	–	–	86.5
0.1	Isoflavone aglycone	51.89 ± 1.68	21.99 ± 0.59	53.67 ± 1.32	127.55 ± 3.59
	Total isoflavone	61.64 ± 0.98	26.23 ± 0.42	64.32 ± 1.02	152.19 ± 2.41
	Conversion rate (%)	–	–	–	83.8

[a] Means ± SD ($n = 3$).
The reaction time was 30 min and the reaction temperature was 55 °C.

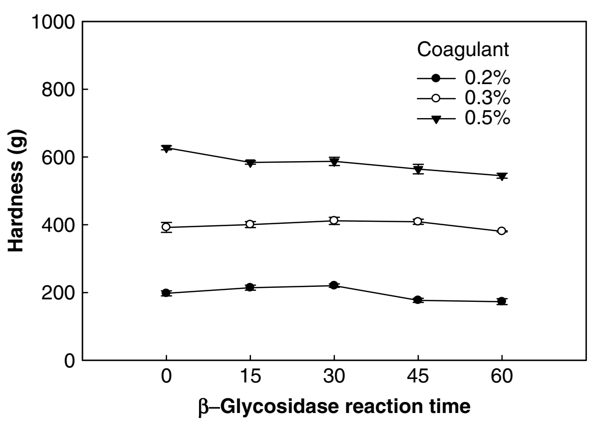

Figure 2. Changes in the hardness of β-glycosidase-treated firm tofu as affected by reaction time and coagulant concentrations. Bars represent standard error of the means ($n = 10$).

colour, surface appearance, taste, texture and overall acceptability of tofu and each attribute had a score above 4, which showed that the enzyme-treated tofu had good overall sensory acceptability and the bio-conversion of isoflavones has no negative effects on the sensory quality of tofu.

Isoflavone content of whey and okara

The pressing step during the manufacture of firm tofu results in the release of whey (a liquid by-product that is discarded), which contains isoflavones; whereas the soft tofu process does not include the pressing step hence no whey is released. The analysis of the isoflavone content of okara (the wet insoluble residue remaining after the filtration process) and whey showed that they contained 25.28 and 22.79 mg of isoflavone per 100 g on a dry basis, respectively (Table 4). Leaching of the isoflavones along with whey during the manufacture of firm tofu has also been reported by Jackson *et al.*[27] Chan *et al.*[28] analysed different soy products for the aglycone forms of daidzein, glycitein and genistein and reported that their contents were higher in soft tofu as compared to firm tofu. The reason is that during soft tofu processing the container is filled with soymilk and sealed before the coagulation process; therefore, isoflavone losses due to whey release are minimised.

Comparison of processing methods for β-glycosidase-treated soft tofu

Isoflavones are biologically valuable polyphenols associated with beneficial disease preventive functions in humans.[29] Many researchers have reported different changes in isoflavones during processing of soybean-based foods.[20] The composition of isoflavones may vary from one step to another during processing.[27] The contents and distribution profile of isoflavones in soybean food products depend on the processing techniques used, such as heat treatment, dilution with non-soy ingredients, fermentation, and enzyme treatment.[22,30] Due to higher contents of isoflavones, we selected soft tofu for the application of two different types of β-glycosidase treatment methods (Fig. 1) in order to evaluate the enzyme efficiency during bio-conversion of isoflavones. In Process 1 (enzyme reaction was carried out before coagulation) 83.4% conversion of isoflavone glycosides to isoflavone aglycones was obtained, whereas Process 2 (enzyme reaction was carried out during

Table 4. Isoflavone contents in tofu by-products

By-product	Type	Isoflavone contents (mg 100 g^{-1} of dried okara or dried whey)[a] Daidzein	Glycitein	Genistein	Total
Okara	Isoflavone aglycone	1.66 ± 0.06	0.28 ± 0.04	2.08 ± 0.07	4.02 ± 0.17
	Total isoflavone	10.05 ± 0.15	1.99 ± 0.04	13.24 ± 0.08	25.28 ± 0.27
Whey	Isoflavone aglycone	0.35 ± 0.01	0.09 ± 0.02	0.26 ± 0.02	0.70 ± 0.01
	Total isoflavone	10.11 ± 1.38	2.31 ± 0.23	10.37 ± 1.47	22.79 ± 3.08

[a] Means ± SD ($n = 3$).

Table 5. Effects of processing methods on the isoflavone contents in soft tofu

Condition	Type	Isoflavone content (mg 100 g^{-1}, dry basis)[a] Daidzein	Glycitein	Genistein	Total
Control	Isoflavone aglycone	8.14 ± 0.65	3.43 ± 0.27	8.21 ± 0.65	19.7 ± 1.50
	Total isoflavone	74.30 ± 0.85	31.48 ± 0.34	76.85 ± 0.88	182.6 ± 1.90
	Conversion rate (%)	–	–	–	10.8
Process 1	Isoflavone aglycone	59.71 ± 0.87	25.30 ± 0.51	61.77 ± 1.39	146.7 ± 2.70
	Total isoflavone	71.59 ± 1.98	30.33 ± 0.66	74.05 ± 1.46	175.9 ± 4.00
	Conversion rate (%)	–	–	–	83.4
Process 2	Isoflavone aglycone	45.74 ± 3.29	19.25 ± 1.38	46.87 ± 3.37	111.8 ± 8.00
	Total isoflavone	71.58 ± 1.20	30.12 ± 0.51	73.34 ± 1.23	175.0 ± 2.90
	Conversion rate (%)	–	–	–	63.9

[a] Means ± SD ($n = 3$).

coagulation) had insufficient enzyme reactions and resulted in only 63.9% conversion as presented in Table 5. It is evident that Process 1 provides sufficient conversions of isoflavones into their aglycone forms using enzyme treatment, first at 55 °C for 30 min and then at a lower temperature (10 °C) as the reaction continues throughout mixing and filling. It has been reported by Manzocco *et al.*[31] that the enzyme reaction can occur at significant rates at lower temperatures. In Process 2, the enzyme reaction is preceded by immediate heating to cause coagulation, which results in a decrease of enzyme activity due to solidification of the reaction medium. The enzyme reaction is controlled by diffusion thus its rate is limited by the increase in viscosity according to the Stokes–Einstein relation.[31] Therefore, Process 2 can be an option when typical tofu production lines are used without any modifications. However, for an optimum β-glycosidase-mediated conversion of glycoside forms of isoflavones to aglycone forms, the induction of enzyme reaction before the onset of coagulation (Process 1) can be recommended. Although tofu making is not a new technology, it is still a challenge to produce tofu with the desired quality characteristics.[32] A complex interaction of many factors including processing conditions occur in making tofu with desired quality attributes such as isoflavones types, hardness and sensory properties. Only by controlling these factors can commercial tofu producers and researchers expect consistency in the resulting product.

CONCLUSIONS

Our study concludes that the use of β-glycosidase during tofu processing is useful for the conversion of glycoside forms of isoflavones to readily bioavailable aglycone forms. Reaction of the enzyme at 55 °C for 30 min using 0.02% β-glycosidase can result in 84.5% conversion. This enzyme-mediated conversion of isoflavones in soft tofu does not have any negative affects on its sensory acceptability. A sufficient hardness of enzyme-treated firm tofu can be obtained by using a coagulant and moderate heating at 55 °C for 30 min during the enzyme reaction. In soft tofu the losses of isoflavones due to whey release are minimised and by incorporating the enzyme reaction before coagulation we can significantly increase the conversion of isoflavones in the final product.

REFERENCES

1 Wang G, Kuan SS, Francis OJ, Ware GM and Carman AS, A simplified HPLC method for the determination of phytoestrogens in soybean and its processed products. *J Agric Food Chem* **38**:185–190 (1990).
2 Potter SM, Baum JA, Teng H, Stillman RJ, Shay NF and Erdman JW, Soy protein and isoflavones: Their effects on blood lipids and bone density in postmenopausal women. *Am J Clin Nutr* **68**:1375–1379 (1998).
3 Adlercreutz H, Phyto-estrogens and cancer. *Lancet Oncol* **3**:364–373 (2002).
4 Messina M, Soyfoods and soybean phyto-estrogens (isoflavones) as possible alternatives to hormone replacement therapy (HRT). *Eur J Cancer* **36**:S71–S77 (2000).
5 Sung CJ, Kim SY, Kim MH and Kim EY, The effect of isoflavone supplementation by soymilk on bone mineral density in underweight college women. *Korean J Nutr* **36**:470–475 (2003).
6 de Kleijn MJ, van der Schouw YT, Wilson PW, Grobbee DE and Jacques PF, Dietary intake of phytoestrogens is associated with a favorable metabolic cardiovascular risk profile in post-menopausal U.S. women: the Framingham study. *J Nutr* **132**:276–282 (2002).
7 Chan YH, Lau KK, Yiu KH, Li SW, Chan HT, Tam S, *et al*, Isoflavone intake in persons at high risk of cardiovascular events: implications for vascular endothelial function and the carotid atherosclerotic burden. *Am J Clin Nutr* **86**:938–945 (2007).

8 Birt DF, Hendrich S and Wang W, Dietary agents in cancer prevention: Flavonoids and isoflavonoids. *Pharmacol Ther* **90**:157–177 (2001).
9 Walter ED, Genistin (an isoflavone glucoside) and its aglycone, genistein, from soybeans. *J Am Chem Soc* **63**:3273–3276 (1941).
10 Ito J, Sahara H, Kaya M, Hata Y, Shibasaki S, Kawata K, *et al*, Characterization of yeast cell surface displayed *Aspergillus oryzae* β-glucosidase 1 high hydrolytic activity for soybean isoflavone. *J Mol Catal B – Enzymes* **55**:69–75 (2008).
11 Bourne MC, *Food Texture and Viscosity Concept and Measurement*. Academic Press, New York, pp. 44–118 (1987).
12 Piskula MK, Yamakoshi J and Iwai Y, Daidzein and genistein but not their glucosides are absorbed from the rat stomach. *FEBS Lett* **447**:287–291 (1999).
13 Izumi T, Piskula MK, Osawa S, Obata A, Tobe K, Saito M, *et al*, Soy isoflavone aglycones are absorbed faster and in higher amounts than their glucosides in humans. *J Nutr* **130**:1695–1699 (2000).
14 Sadler MJ, Meat alternatives – market developments and health benefits. *Trends Food Sci Tech* **15**:250–260 (2004).
15 Watanabe T, *Science of Tofu*. Food Journal Co. Ltd., Kyoto, pp. 8–63 (1997).
16 Kurzer MS and Xu X, Dietary phytoestrogens. *Annu Rev Nutr* **17**:353–381 (1997).
17 Pyo YH, Lee TC and Lee YC, Enrichment of bioactive isoflavones in soymilk fermented with β-glucosidase-producing lactic acid bacteria. *Food Res Int* **38**:551–559 (2005).
18 Hou HJ, Chang KC and Shih MC, Yield and textural properties of soft tofu as affected by coagulation method. *J Food Sci* **62**:824–827 (1997).
19 Fukutake M, Takahashi M, Ishida K, Kawamaru H, Sugimura T and Wakabayashi K, Quantification of genistein and genistin in soybeans and soybean products. *Food Chem Toxicol* **34**:457–461 (1996).
20 Coward L, Smith M, Kirk M and Barnes S, Chemical modification of isoflavones in soyfoods during cooking and processing. *Am J Clin Nutr* **68**:1486–1491 (1998).
21 Ismail B and Hayes K, β-Glycosidase activity toward different glycosidic forms of isoflavones. *J Agric Food Chem* **53**:4918–4924 (2005).
22 Ishihara M, Singh H, Chung G and Tam C, Content composition and antioxidant activity of isoflavones in commercial and homemade soymilk and tofu. *J Sci Food Agric* **87**:2844–2852 (2007).
23 Noh EJ, Park SY, Pak JI, Hong ST and Yun, SE, Coagulation of soymilk and quality of tofu as affected by freeze treatment of soybeans. *Food Chem* **91**:715–721 (2005).
24 Liu ZS, Chang SKC, Li LT and Tatsumi E, Effect of selective thermal denaturation of soybean proteins on soymilk viscosity and tofu's physical properties. *Food Res Int* **37**:815–822 (2004).
25 Prabhakaran MP, Perera CO and Valiyaveettil S, Effect of different coagulants on the isoflavone levels and physical properties of prepared firm tofu. *Food Chem* **99**:492–499 (2006).
26 Saio K, Tofu – relationships between texture and fine structure. *Cereal Food World* **24**:342–354 (1979).
27 Jackson CJC, Dini JP, Lavandier C, Rupasinghe HPV, Faulkner H, Poysa V, *et al*, Effects of processing on the content and composition of isoflavones during the manufacturing of soy beverage and tofu. *Process Biochem* **37**:1117–1123 (2002).
28 Chan SG, Murphy PA, Ho SC, Kreiger N, Darlington D, So EKF, *et al*, Isoflavonoid content of Hong Kong soy foods. *J Agric Food Chem* **57**:5386–5390 (2009).
29 Barbosa ACL, Lajolo FM and Genovese MI, Influence of temperature, pH and ionic strength on the production of isoflavone-rich soy protein isolates. *Food Chem* **98**:757–766 (2006).
30 Wang J and Murphy PA, Mass balance study of isoflavones during soybean processing. *J Agric Food Chem* **44**:2377–2383 (1996).
31 Manzocco L, Nicoli MC, Anese M, Pitottia A and Maltini E, Polyphenoloxidase and peroxidase activity in partially frozen systems with different physical properties. *Food Res Int* **31**:363–370 (1999).
32 Cai TD, Chang KC, Shih MC, Hou HJ and Ji M, Comparison of bench and production scale methods for making soymilk and tofu from 13 soybean varieties. *Food Res Int* **30**:659–668 (1997).

