Journal of Food and Nutrition Research, 2015, Vol. 3, No. 9, 613-619
Available online at http://pubs.sciepub.com/jfnr/3/9/9

DOI:10.12691/jfnr-3-9-9

Protease Treatment, Glucose Addition and Saccharification of Adzuki Beans Effects on the Radical-scavenging Properties of Soymilk

Shuo Feng[1,2], Shan W[1,2], Michiyuki Kojima[1,*]

[1]Department of Food Production Science, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido, Japan
[2]United Graduate School of Agricultural Sciences, Iwate University, Ueda, Morioka, Iwate, Japan
*Corresponding author: kojima@obihiro.ac.jp

Abstract The effects of protease treatment, glucose addition and adzuki beans saccharification on the radical-scavenging properties of soymilk were evaluated. It was found that protease treatment (8.9 U/ml) and the glucose addition (84 mg/ml) increased the radical-scavenging capacity of soymilk by 1.8 folds. Adzuki bean saccharification (51 U/ml α-amylase and 6.25 U/ml amyloglucosidase) and protease treatment (8.9 U protease/ml) increased radical-scavenging activity by 2.3 folds. This effect was not solely due to the presence of adzuki polyphenols, as melanoidin production, but also due to increased radical-scavenging activity. The correlation between the radical-scavenging capacity of soymilk and melanoidins, or that of mix bean milk and melanoidins was high (soymilk: $R^2 = 0.94$; mix bean: $R^2 = 0.96$). Collectively, these data indicated that protease treatment, glucose addition and adzuki bean milk saccharification affect the radical-scavenging activity of soymilk through melanoidin production. These findings should contribute to the development of soymilk-based antioxidant-rich functional foods formulation in the future.

Keywords: *soymilk, functional food, radical-scavenging activity, antioxidant activity, proteolytic treatment, saccharification, maillard reaction; melanoidins*

Cite This Article: Shuo Feng, Shan W, and Michiyuki Kojima, "Protease Treatment, Glucose Addition and Saccharification of Adzuki Beans Effects on the Radical-scavenging Properties of Soymilk." *Journal of Food and Nutrition Research*, vol. 3, no. 9 (2015): 613-619. doi: 10.12691/jfnr-3-9-9.

1. Introduction

Soybeans are native to Asia, and soymilk is a popular beverage that provides proteins, dietary fiber, low cholesterol content, lactose-free quality and high levels of bioactive phenolic compounds [1,2]. The worldwide consumption of soymilk has steadily grown in recent years, although some countries (such as China) have a low per-capita consumption rate; furthermore, functional foods made from soybeans have attracted widespread interest. Functional foods prepared from soybeans are rich sources of non-digestible galactooligosaccharides, dietary soybean protein, isoflavone and antioxidants [3,4,5]. It was reported that the fermentation of soybean products eliminated trypsin inhibitors and increased the amount of small-sized peptides [6]. However, relatively little research has been conducted in terms of improving the antioxidant content of soybean-based foods.

Free radicals are highly reactive and potentially damaging, transient chemical species that are formed in aerobic organisms [7]. Free-radical production can result from cell membrane disintegration, membrane protein damage, and DNA mutations and can increase the risks for developing several chronic human diseases such as cancer, arteriosclerosis, and neurodegenerative disorders and can promote aging processes [8,9]. Antioxidants can mitigate or eliminate related oxidative damage and guard against the development of some diseases like atherosclerosis, cancer, and senility resulting from bioactive substances, such as polyphenolic compounds and melanoidins [10,11,12,13]. The Maillard reaction (MR) is classified as a non-enzymatic browning reaction, which is a very complex reaction that occurs between carbonyl-containing compounds such as reducing sugars, aldehydes or ketones and amino-containing compounds such as amino acids, proteins or other nitrogenous compounds [14]. Many studies have reported beneficial effects associated with Maillard reaction products (MRPs), including radical chain-breaking activity [15,16] and reducing power [17,18], and that glucose undergoes a quicker MR with lysine than with arginine or tryptophan. Soybeans are rich in lysine; thus, during soymilk processing, the production of antioxidants such as melanoidins via MRs has been predicted. Adzuki beans are rich in polyphenols such as quercetin glycoside and proanthocyanidins, which have been used as natural antioxidants [19,20].

Adzuki bean solution (raw adzuki bean milk), which contains adzuki bean starch, cannot be directly added to beverages because of the gelatinization of starch. Usually, this problem can be overcome by instead adding the essence of adzuki to beverages, or through starch liquefaction. Therefore, during soymilk processing, the production of polyphenols having antioxidative activity from adzuki beans has been expected. The purpose of this

study was to test the hypothesis that the radical-scavenging activity of soymilk-based functional foods can be improved through protease treatment, glucose addition and the saccharification of adzuki beans. Increasing the radical-scavenging activities of food products will aid in developing functional foods that are of benefit to human health.

2. Materials and Methods

2.1. Materials

Soybean and adzuki beans were harvested in Hokkaido, Japan. The mycolysin protease (EC number 3.4.24.31) was purchased from Wako Pure Chemical Industries, Ltd. (Japan). α-Amylase from *Bacillus* species (EC number 3.2.1.1) and amyloglucosidase from *Aspergillus niger* (EC number 3.2.1.3) were purchased from Sigma-Aldrich, Inc. (Japan). The free radical compound 1-diphenyl-2-picrylhydrazyl (DPPH) was purchased from Tokyo Chemical Industry, Co., Ltd. All other purchased chemicals were of analytical grade.

2.2. Methods

2.2.1. Milk Preparation

Soybeans (50 g) were placed into a pot and soaked in 300 ml deionized water at 25°C for 16 h. Subsequently, the immersed soybeans and water were ground to raw milk in a mixer (JE-223c; Sun Co., Ltd., Japan) for 1 min, after which the raw milk was placed into a milk detritus-separating machine (100 mesh, MLBG-2001; Tokyo Unicom Co., Ltd., Japan). Raw adzuki bean milk was prepared by soaking adzuki beans (50 g) in 100 mL deionized water for 16 h in a covered pot at 25°C. Next, the immersed adzuki beans and water were separated using a sieve. The volume of immersion water was measured, and then the skin was peeled off the adzuki beans. Before mixing, fresh deionized water (i.e., the measured "immersion volume" + 200 mL additional water) was added to the adzuki beans. After mixing for 1 min, the raw milk was placed into a milk detritus-separating machine. The separated soymilk was immediately treated with mycolysin protease, whereas the raw adzuki bean milk was directly used in liquefaction and saccharification experiments. Boiled, raw milk was prepared by first placing 40 ml soymilk or a 1:1 (v:v) mixture of soymilk and adzuki bean milk into a 500-mL flask (TE-32; Iwaki AGC Techno Glass Co., Ltd., Japan), sealing it with aluminum foil and locking the aluminum foil into place with an elastic band. The milk was boiled at 100°C for 10 min using a MAG-Mixer MH-61 heater (Yamato scientific Co., Ltd., Japan) and mixed with a magnetic stirrer. Next, the flask was washed using deionized water, which was also used to adjust the volume to 80 ml. Finally, the milk was homogenized (26×g, 30 s, ASN-5-4039-01; AS One Corporation, Japan) and used for subsequent experiments.

2.2.2. Protease Treatment of Raw Soymilk

For hydrolysis studies, raw homogenized soymilk (40 ml) was mixed with differing numbers of units of protease, transferred into screw-top 50-ml test tubes, and incubated in a shaking water bath set at 160 rpm (Personal-11; Taitec Co., Ltd., Japan) at 58°C for 1 h. Subsequently, the raw milk was immediately boiled, or the free amino-group and soluble protein concentrations were measured.

2.2.3. Liquefaction and Saccharification of raw Adzuki Bean Milk

Raw adzuki bean milk (100 ml) was gelatinized by placing it in a beaker and transferring the beaker to a heater set at 100°C for 10 min, with magnetic stirring. Next, the volume was adjusted to 100 ml by deionized water and then mixed with α-amylase (51 U/ml) for liquefaction. The adzuki bean solution (40 ml) was put in a 50-ml screw-top test tube, and liquefaction was achieved by heating it in a water bath shaker (160 rpm) for 30 min at 60°C. For saccharification, liquefacted, raw adzuki bean milk (40 ml) was mixed with amyloglucosidase solutions with differing activities; 0.1 ml of the amyloglucosidase solution will digest 1 g of corn starch to glucose, and this activity was defined as 1 U/μl. Next, the liquefacted raw milk was incubated at 55°C for 120 min in a water bath shaker set at 160 rpm. The treatment solution of raw adzuki bean was immediately used for the preparation of milk, or measuring the reducing sugar and glucose content after enzymatic treatment.

2.2.4. Measurements of DPPH Radical-scavenging Activities

The method of Blois [21] was used in this study. Briefly, each milk sample was appropriately diluted by deionized water (soymilk was diluted by 8 or 16 folds; adzuki bean milk and adzuki bean milk mixed with soymilk were diluted by 40 folds). In each case, the diluted sample (0.75 ml) was mixed with an equal volume 2 mM DPPH dissolved in ethanol, fully shaken, kept in the dark for 15 min reaction at room temperature and centrifuged at 13,700×g for 15 min at 5°C. Subsequently, the absorbance at 517 nm was measured using a 96-well microplate reader. Standard curves were constructed using increasing concentrations of trolox (0–30 nM). The antioxidant activities of samples were therefore expressed in terms of trolox equivalents (nM).

2.2.5. Preparation and Measurement of Melanoidins

The method of Martins & van Boekel [22] was used with minor modifications to prepare melanoidins. We used a final concentration of 0.2 M of reaction solution, which contained equimolar amounts of glucose and glycine mixed with 0.1 M phosphate (pH 6.8) to a volume of 100 ml. This solution was distributed into 10 screw-top glass tubes, each tube containing 10 ml. The samples were heated at 120°C for 2 h, after which they were cooled on ice and dialyzed. As the dialyzed procedure, twenty milliliters of the reaction solution was placed into a dialysis membrane (14,000 molecular-weight cutoff; UC 36-32-100; EIDIA Co., Ltd., Japan) and dialyzed against deionized water (2 L) for 9 days. The deionized water was changed twice per day, until it was nearly colorless following dialysis. The reaction solution was immediately frozen at –20°C for 12 h and then freeze-dried for 48 h. Next, the powder was weighed and dissolved in 10 ml deionized water to construct standard curves (0–100 μg dissolved powder/ml). Distilled water without dissolved

powder was used as a blank for determining absorption values at 450 nm. Samples of milk were mixed with ethanol (1:1), fully shaken, and centrifuged at 13,700×g (MX-150; Tomy Seiko Co., Ltd., Japan) for 15 min at 5°C, after which absorbance of the supernatant was measured at 450 nm with a U-1800 spectrophotometer (Hitachi High-Tech Science Corporation, Japan). To reduce the effects of pigments present on melanoidin production, we measured the absorbance of melanoidins to determine the absorbance of boiled soymilk and adzuki milk, which were not subjected to enzyme treatment. Then, we generated standard curves to compute the content of melanoidins.

2.2.6. Total Polyphenol Content Measurements

Total polyphenol content was determined using the Folin–Ciocalteu method [23]. A calibration curve (0 to 62.5 μg catechin/ml) was prepared, and the results are expressed as mg catechin acid equivalents/g in each sample. Using this method, the samples were mixed with ethanol (1:1), fully shaken, and centrifuged at 13,700×g for 15 min at 5°C, after which the supernatants were appropriately diluted by deionized water (soymilk was diluted 2 folds; both adzuki bean milk and adzuki bean milk mixed with soymilk were diluted 8 folds). In each case, 0.4 ml of diluted sample was mixed with 0.4 ml of 50% Folin reagent, shaken thoroughly and allowed to stand for 3 min. Next, 0.4 ml 10% sodium carbonate solution was added to each mixture, which was thoroughly shaken and incubated for 30 min at 30°C, followed by centrifugation at 900×g for 10 min. The absorbance at 760 nm was then measured using a spectrophotometer.

2.2.7. Measuring the Concentration of Soluble Proteins

Soluble milk protein concentrations were measured using the Bradford method [24]. Briefly, milk samples were centrifuged at 900×g for 10 min, and the supernatant was diluted 30 folds with deionized water. The supernatant (20 μl) was mixed with deionized water (30 μl) and Bradford reagent (1 ml) and thoroughly shaken. The absorbance was measured at 595 nM using a spectrophotometer, with the control sample (50 μl deionized water and 1 ml Bradford reagent) being set to zero. Bovine serum albumin was used as a standard.

2.2.8. Measurements of Free Amino-group Content

Free amino-group content was determined by performing ninhydrin reactions. Each sample was mixed with ethanol (1:1), fully shaken, centrifuged at 13,700×g for 15 min at 5°C, and the supernatant was diluted 10 folds in deionized water. Next, 0.1 ml of diluted sample was mixed with 0.1 ml of sodium acetate buffer (pH = 5.4), ninhydrin solution (5.3 mg/ml in ethanol; 0.3 ml), and 0.01 ml of 3% ascorbic acid; thoroughly shaken; heated at 100°C for 15 min; and cooled on ice. Subsequently, the sample volumes were adjusted to a constant volume of 2 mL in 60% ethanol. The absorbance values of the blue-purple ninhydrin reaction products were measured at 570 nm, against a reagent blank. Glycine was used as a standard.

2.2.9. Measurement of Reducing Sugar Content

The concentrations of reducing sugars were determined using the 3,5-dinitrosalicylic acid (DNS) method, [25] with glucose serving as a calibration standard. This method is based on the reaction of DNS with reducing sugars during which DNS acid is formed under alkaline conditions. The concentrations of reducing sugars in samples were determined by taking absorbance measurements at 540 nm and interpolation with pre-constructed calibration curves. The samples were mixed with ethanol (1:1), fully shaken, and centrifuged at 13,700×g for 15 min at 5°C, after which the supernatant was appropriately diluted with deionized water. RA and RamA625 were analyzed in undiluted form, whereas RaA, RaamA025, RaamA125, and RaamA625 were diluted 8 folds.

2.2.10. Measurement of Glucose Content

Glucose content was determined using a portable glucose meter (GF-501-H; Tanita Co., Ltd., Japan). Glucose concentrations in solution were measured via enzymatic glucose oxidase reactions, with glucose serving as a calibration standard. Milk samples were centrifuged at 900×g for 10 min, and the supernatant was appropriately diluted with deionized water. In these experiments, the glucose content was measured in (i) undiluted RA and RamA625, or (ii) 3 folds diluted RaA, RaamA025, RaamA125, and RaamA625 that were diluted by 20 folds.

2.2.11. Statistical Analysis

Statistical analysis was performed to the obtained results with all bean milk samples, using two different batches of milk. All measurements were obtained in triplicate. The values are expressed as the arithmetic means. T testing (least squares differences) was performed to identify significant differences among the analyzed samples and differences were considered statistically significant at $p < 0.05$.

3. Results and Discussion

3.1. Effect of Protease and Glucose Addition on the DPPH Radical-scavenging Activity of Soymilk

To investigate the effects of protease and glucose addition on the DPPH radical-scavenging activity of soymilk, protease was added at three concentrations (0, 2.2, and 8.9 U/mL), and glucose was added at concentrations ranging from 0–84 mg/ml. Figure 1 shows that when protease and glucose were simultaneously added, the level of radical-scavenging activity significantly increased. Radical-scavenging activities was the greatest and being 1.8 folds greater than that in soymilk without added when the utilized glucose and protease were 8.9 U/ml and glucose 84 mg/ml, respectively.

These results may reflect an increased production of glucose molecules and free amino-groups liberated by enzymatic digestion. Total protein concentrations decreased in parallel with the increase in free amino-groups when raw soymilk was treated by protease digestion (Table 1). It may due to that it breaks down long chains of proteins into polypeptide and shorter free amino acids [26,27]. However, no significant difference in DPPH radical-scavenging activity of soymilk was observed when the amount of glucose added was increased from 56 mg/ml (Bp2S, Bp8S) to 84 mg/ml (Bp2S, Bp8S). This result may have been caused by limitations in the reaction

conditions, such as the heating time or heating temperature [28]. To avoid losses of flavor or nutrition in the soymilk, caused by excessive heating, the heating time was limited to 10 minutes, and the heating temperature was 100°C. Without protease treatment, glucose addition also caused a small, but significant, increase in radical-scavenging activity. This result was potentially due to baseline MR activity with the added glucose and the small amount of free amino-groups present in soybean liquid. It was reported that glucose addition can cause caramelization and increase radical-scavenging activity [29]. However, in our experiments, when we increased the added glucose concentration from 28 mg/ml to 84 mg/ml, no significant increase in radical-scavenging activity was observed. The temperature and time of heating can affect caramelization [30,31]. Without the addition of glucose, protease treatment also caused a significant increase in radical-scavenging activity, as shown in Figure 1. It was reported that when soybean proteins were treated by proteolytic digestion, the radical-scavenging activity increased [32]. We also investigated changes in the total polyphenol content of soymilk following protease treatment (Table 2). Although an increased value was observed following protease treatment, this may not reflect a true increase in total polyphenols, because the measurements were performed using the Folin reagent, which can react with proteins and cause absorbance at 750 nm [33]. Thus, this reagent may have reacted with the free amino-groups liberated by protease treatment. No significant differences between the effects of Bp2S and Bp8S on DPPH radical-scavenging capacity were observed, in cases where glucose was not added.

Table 1. Changes in free amino-group content and protein concentrations in raw soybean milk following protease treatment

	Free amino group			Soluble protein concentration		
	(mg/ml)			(%)		
RS*	0.75^{c}	±	0.02	4.65^{a}	±	0.08
Rp2S*	1.62^{b}	±	0.28	3.41^{b}	±	0.07
Rp8S*	2.77^{a}	±	0.19	2.97^{c}	±	0.09

*: The description of samples is provided in Figure 1; Superscripted letters in the vertical line indicate significant difference (p <0.05)

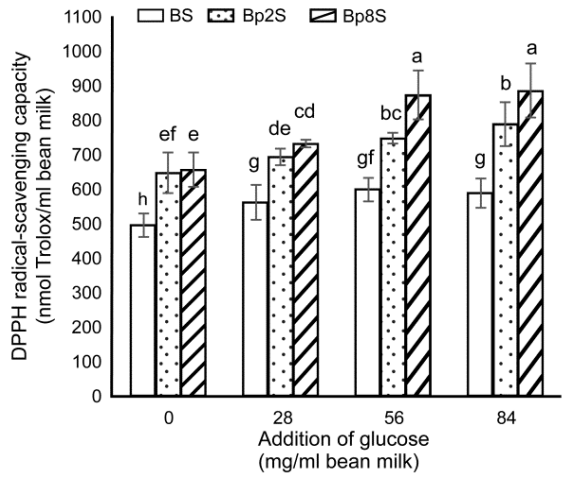

Figure 1. Radical-scavenging capacity of 1-diphenyl-2-picrylhydrazyl (DPPH) in boiled soymilk following glucose and/or protease addition (p <0.05); BS: boiled soybean milk not subjected to protease treatment; Bp2S: boiled soybean milk treated with 2.2 U/ml protease; Bp8S: boiled soybean milk treated with 8.9 U/ml protease

Table 2. Changes in total polyphenol content of boiled soybean milk, boiled azuki bean milk, and boiled mixed-bean milk following enzyme treatment

	Total polyphenol		
	(mg/ml)		
Soybean milk*			
BS	0.53^{c}	±	0.02
Bp2S	0.67^{b}	±	0.05
Bp8S	0.84^{a}	±	0.06
adzuki bean milk			
BA	0.25	±	0.02
BaA	0.25	±	0.01
BamA625	0.25	±	0.03
BaamA625	0.24	±	0.02
Mixed-bean milk**			
BSA	0.37^{b}	±	0.01
Bp8SaA	0.60^{a}	±	0.03
Bp8SamA625	0.61^{a}	±	0.04
Bp8SaamA025	0.61^{a}	±	0.03
Bp8SaamA125	0.62^{a}	±	0.03
Bp8SaamA625	0.61^{a}	±	0.03

*: The description of samples is provided in Figure 1; BA: boiled adzuki bean milk not subjected to enzyme treatment; BaA: boiled adzuki bean milk treated with 51 U/ml α-amylase; BamA625: boiled adzuki bean milk treated with 6.25 U/ml amyloglucosidase; BaamA625: boiled adzuki bean milk treated with 51 U/ml α-amylase and 6.25 U/ml amyloglucosidase; **: The description of samples is provided in Figure 2. (A); Superscripted letters in the vertical line indicate significant difference (p <0.05)

3.2. Effect of the Saccharification of Adzuki Bean Milk on the DPPH Radical-scavenging Activity of Soymilk

Table 3. Changes in reducing sugar and glucose content in raw adzuki bean milk following enzyme treatment

	Reducing sugar content			Glucose content		
	(mg/ml)			(mg/ml)		
RA	1.89^{e}	±	0.10	0.49^{e}	±	0.08
RaA	70.78^{d}	±	4.92	7.66^{d}	±	0.36
RamA625	2.00^{e}	±	0.11	1.01^{e}	±	0.06
RaamA025	92.94^{c}	±	2.00	55.47^{c}	±	4.14
RaamA125	103.99^{b}	±	2.39	71.00^{b}	±	3.19
RaamA625	119.36^{a}	±	6.96	91.63^{a}	±	3.86

RA: raw adzuki bean milk not subjected to enzyme treatment; RaA: raw adzuki bean milk treated with 51 U/ml α-amylase; RamA625: raw adzuki bean milk treated with 6.25 U/ml amyloglucosidase; RaamA025: raw adzuki bean milk treated with 51 U/ml α-amylase and 0.25 U/ml amyloglucosidase; RaamA125: raw adzuki bean milk treated with 51 U/ml α-amylase and 1.25 U/ml amyloglucosidase; RaamA625: raw adzuki bean milk treated with 51 U/ml α-amylase and 6.25 U/ml amyloglucosidase; Superscripted letters in the vertical line indicate significant difference (p <0.05)

Figure 2A shows that Bp8SaamA625, Bp8SaamA125, and Bp8SaamA025 mixed-bean milk had higher radical-scavenging activity than did Bp8SamA625, Bp8SaA, and BSA bean milk, which were subjected to amyloglucosidase treatment only, α-amylase treatment only, or no enzyme treatment, respectively. In addition, by varying the

concentrations of amyloglucosidase added to the three mixed-bean milks, we observed that amyloglucosidase treatment caused a dose-dependent increase in radical-scavenging activity. This result was due to an increase in reducing sugar content, especially that of glucose, which can react with free amino-groups to generate active products. Figure 2B shows that there was no increase in radical-scavenging activity between BaamA625 and BA, which might be due to a low content of free amino-groups in adzuki bean milk not subjected to protease treatment. Table 3 shows the effects of reducing sugar and glucose content. A distinct increase in the content of these 2 sugars was observed with increasing amyloglucosidase concentrations. In addition, the raw adzuki bean milk sample treated only with raw α-amylase (RaA) had a high reducing sugar content, but glucose only detected a comprised 11% of the reducing sugars, and the glucose content was much lower than that for the three mixed-bean milks.

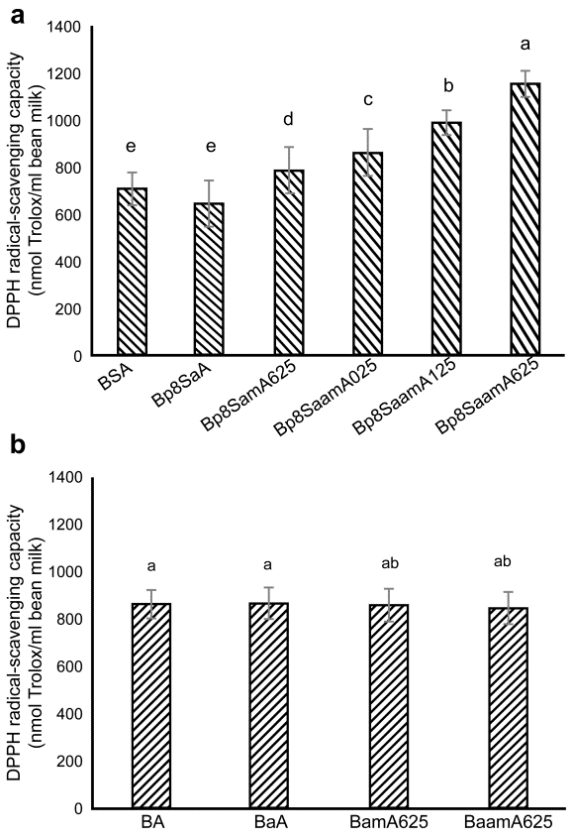

Figure 2. (A) Radical-scavenging capacity of 1-diphenyl-2-picrylhydrazyl (DPPH) in boiled mixed-bean milk (p <0.05); BSA: boiled bean milk, raw soybean milk, and adzuki bean milk not subjected to enzyme treatment; Bp8SaA: boiled bean milk and raw soybean milk were treated with 8.9 U/ml protease, and raw adzuki bean milk was treated with 51 U/ml α-amylase; Bp8SamA625: boiled bean milk and raw soybean milk were treated with 8.9 U/ml protease, and raw adzuki bean milk was treated with 6.25 U/ml amyloglucosidase; Bp8SaamA025: boiled bean milk and raw soybean milk were treated with 8.9 U/ml protease, and raw adzuki bean milk was treated with 51 U/ml α-amylase and 0.25 U/ml amyloglucosidase; Bp8SaamA125: boiled bean milk and raw soybean milk were treated with 8.9 U/ml protease, and raw adzuki bean milk was treated with 51 U/ml α-amylase and 1.25 U/ml amyloglucosidase; Bp8SaamA625: boiled bean milk and raw soybean milk were treated with 8.9 U/ml protease, and raw adzuki bean milk was treated with 51 U/ml α-amylase and 6.25 U/ml amyloglucosidase; (B) DPPH radical-scavenging capacity of boiled adzuki bean milk (p <0.05); The description of samples is provided in Table 2

No significant differences were observed between these three samples in terms of their total polyphenol content. In addition, no significant differences were observed between the total polyphenol content of adzuki bean milks (Table 2). These findings indicated that the observed increases of radical-scavenging activity were not dependent on polyphenols. A lower total polyphenol content was also found in BSA bean milk than in other mixed-bean milk, which was similar to the changes observed in soymilk following protease treatment. These results may have reflected measurement errors caused by the Folin reagent. However, no significant differences between Bp8SaamA025, Bp8SaamA125, and Bp8SaamA625 in polyphenol values, but showed increased radical-scavenging activities. Yoshimura et al. [34] reported that MRPs formed using the glucose–glycine model system had a greater ability to scavenge hydroxide radicals. Our results suggested that the MRPs caused significant differences in radical-scavenging activities between Bp8SaamA625 and BSA.

3.3. Correlations between Soymilk and Mixed-bean Milk with DPPH Radical-scavenging Activity and Melanoidin Production

Figure 3 shows that high correlations were observed between soymilk and mixed-bean milk in terms of the associated levels of DPPH radical-scavenging activity and melanoidins. These results are similar to those of Liu et al., [35] who used a brown pigment to measure the melanoidins, showing an association between DPPH activity and brown pigment. It was also found that the radical-scavenging activity of mixed-bean milk was higher than that of soymilk, due to the high content of polyphenols.

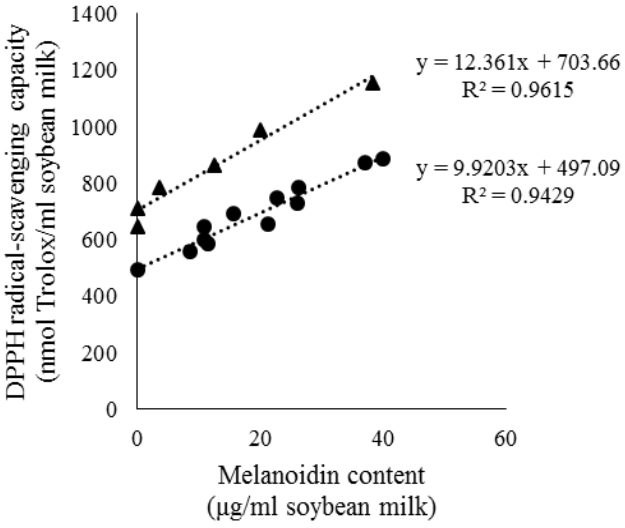

Figure 3. Correlation coefficients between the melanoidin content and 1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging capacity. Triangles, mixed bean milk; circles, soymilk

4. Conclusions

Protease treatment, glucose addition, and the saccharification of adzuki bean milk can affect the radical-scavenging activity of soymilk through the production of melanoidins. These findings should contribute to the

development of soymilk-based, antioxidant-rich functional foods in the future.

Acknowledgement

The authors wish to thank Professor T. Owada at Obihiro University of Agriculture and Veterinary Medicine, Professor E. Sugawara and Professor C. Tsukamoto of Iwate University, for helpful scientific discussions.

Statement of Competing Interests

The authors have no competing interests.

List of Nonstandard Abbreviations

A: adzuki bean milk; a: α-amylase; am: amyloglucosidase; B: boiled; MR: Maillard reaction; MRPs: Maillard reaction products; p: protease; R: raw; S: soymilk

References

[1] Chen, K.I., Erh, M.H., Su, N.W., Liu, W.H., Chou, C.C. and Cheng, K.C., "Soyfoods and soybean products: from traditional use to modern applications," *Applied Microbiology and Biotechnology*, 96 (1). 9-22. Oct. 2012.

[2] Ma, L., Li, B., Han, F., Yan, S., Wang, L. and Sun, J, "Evaluation of the chemical quality traits of soybean seeds, as related to sensory attributes of soymilk," *Food Chemistry*, 173. 694-701. Apr. 2015.

[3] Arjmandi, B.H., Alekel L., Hollis B.W., Amin D., Stacewicz-Sapuntzakis M., Guo P, and Kukreja, S.C., "Dietary soybean protein prevents bone loss in an ovariectomized rat model of osteoporosis," *Journal of Nutrition*, 126 (1). 161-167. Jan. 1996.

[4] Devi, M.K.A., Gondi, M., Sakthivelu, G., Giridhar, P., Rajasekaran, T. and Ravishankar, G.A., "Functional attributes of soybean seeds and products, with reference to isoflavone content and antioxidant activity," *Food Chemistry*, 114 (3). 771-776. Jun. 2009.

[5] Espinosa-Martos, I. and Rupérez-Antón, P., "Soybean oligosaccharides. Potential as new ingredients in functional food," *Nutrición Hospitalaria*, 21 (1). 92-96. Nov. 2006.

[6] Hong, K.J., Lee, C.H. and Kim, S.W, "*Aspergillus oryzae* GB-107 fermentation improves nutritional quality of food soybeans and feed soybean meals," *Journal of Medicinal Food*, 7 (4). 430-435. Dec. 2004.

[7] Wang, J., Yuan, X., Jin, Z., Tian, Y. and Song, H, "Free radical and reactive oxygen species scavenging activities of peanut skins extract," *Food Chemistry*, 104 (1). 242-250. Jan. 2007.

[8] Aruoma, O.I., "Free radicals, oxidative stress, and antioxidants in human health and disease," *Journal of the American Oil Chemists' Society*, 75 (2). 199-212. Feb. 1998.

[9] Valentão, P., Fernandes, E., Carvalho, F., Andrade, B.P., Seabra, R.M. and de Lourdes Bastos, M, "Antioxidant activity of *Hypericum androsaemum* infusion: scavenging activity against superoxide radical, hydroxyl radical and hypochlorous acid," *Biological and Pharmaceutical Bulletin*, 25 (10). 1320-1323. Oct. 2002.

[10] Ani, V., Varadaraj, M.C. and Akhilender Naidu, K, "Antioxidant and antibacterial activities of polyphenolic compounds from bitter cumin (*Cuminum nigrum* L.)," *European Food Research and Technology*, 224. 109-115. Mar. 2006.

[11] Gülçın, İ, Oktay, M, Kıreççı, E and Küfrevıoğlu, Ö.İ., "Screening of antioxidant and antimicrobial activities of anise (*Pimpinella anisum* L.) seed extracts," *Food Chemistry*, 83 (3). 371-382. Apr. 2003.

[12] Valls-Bellés, V., Torres, M.C., Muñiz, P., Boix, L., González-Sanjose, M.L. and Codoñer-Franch, P., "The protective effects of melanoidins in adriamycin-induced oxidative stress in isolated rat hepatocytes," *Journal of the Science of Food and Agriculture*, 84 (13). 1701-1707. Aug. 2004.

[13] Esposito, F., Morisco, F., Verde, V., Ritieni, A., Alezio, A., Caporaso, N. and Fogliano, V., "Moderate coffee consumption increases plasma glutathione but not homocysteine in healthy subjects," *Alimentary Pharmacology and Therapeutics*, 17 (4). 595-601. Feb. 2003.

[14] Liu, J., Ru, Q. and Ding, Y., "Glycation a promising method for food protein modification: physicochemical properties and structure, a review," *Food Research International*, 49 (1). 170-183. Jul. 2012.

[15] Hwang, I.G., Kim, S.H., Woo, K.S., Lee, J. and Jeong, H.S., "Biological activities of Maillard reaction products (MRPs) in a sugar–amino acid model system," *Food Chemistry*, 126 (1). 221-227. May 2011.

[16] Morales, F.J. and Jiménez-Pérez, S, "Free radical scavenging capacity of Maillard reaction products as related to colour and fluorescence," *Food Chemistry*, 72 (1). 119-125. Jan. 2001.

[17] Gu, F., Kim, J.M., Hayat, K., Xia, S., Feng, B. and Zhang, X, "Characteristics and antioxidant activity of ultrafiltrated Maillard reaction products from a casein–glucose model system," *Food Chemistry*, 117 (1). 48-54. Nov. 2009.

[18] Yen, G.C., Tsai, L.C. and Lii, J.D., "Antimutagenic effect of Maillard browning products obtained from amino acids and sugars," *Food and Chemical Toxicology*, 30 (2). 127-132. Feb. 1992.

[19] Ariga, T. and Hamano, M, "Radical scavenging action and its mode in procyanidins B-1 and B-3 from azuki beans to peroxyl radicals," *Agricultural and Biological Chemistry*, 54 (10). 2499-2504. Sep. 1990.

[20] Yoshida, Y., Kondo, T., Ito, M. and Kondo, T, "Analysis of polyphenols in water extract of red adzuki bean, *Vigna angularis*," *ITE Letters*, 6, 226-231. 2005.

[21] Blois, M.S., "Antioxidant determinations by the use of a stable free radical," *Nature*, 181, 1199-1200. Apr. 1958.

[22] Martins, S.I.F.S. and van Boekel, M.A.J.S., "Melanoidins extinction coefficient in the glucose/glycine Maillard reaction," *Food Chemistry*, 83 (1). 135-142. Oct. 2003.

[23] Rakića, S., Petrović, S., Kukić, J., Jadraninc, M., Tešević, V., Povrenović, D. and Šiler-Marinković, S., "Influence of thermal treatment on phenolic compounds and antioxidant properties of oak acorn from Serbia," *Food Chemistry*, 104 (2). 830-834. Jan. 2007.

[24] Seevaratnam, R., Patel, B.P. and Hamadeh, M.J., "Comparison of total protein concentration in skeletal muscle as measured by the Bradford and Lowry assays," *Journal of Biochemistry*, 145 (6). 791-797. Jun. 2009.

[25] Miller, G.L., "Use of dinitrosalicylic acid reagent for determination of reducing sugar," *Analytical Chemistry*, 31 (3). 426-428. Mar. 1959.

[26] Socha, P., Mickowska, B., Urminská, D. and Kačmárová, K., "The use of different proteases to hydrolyze gliadins," *Journal of Microbiology, Biotechnology and Food Sciences*, 4 (2). 101-104. Feb. 2015.

[27] Frackenpohl, J., Arvidsson, P.I., Schreiber, J.V. and Seebach, D., "The Outstanding Biological Stability of *β*- and *γ*-Peptides toward Proteolytic Enzymes: An In Vitro Investigation with Fifteen Peptidases," *ChemBioChem*, 2 (6). 445-455. May 2001.

[28] Liu, G. and Zhong, Q, "High temperature-short time glycation to improve heat stability of whey protein and reduce color formation," *Food Hydrocolloids*, 44, 453-460. Feb. 2015.

[29] Tsai, P.J., Yu, T.Y., Chen, S.H., Liu, C.C. and Sun, Y.F., "Interactive role of color and antioxidant capacity in caramels," *Food Research International*, 42 (3). 380-386. Apr. 2009.

[30] Homoki-Farkas, P., Orsi, F. and Kroh, L.W., "Methylglyoxal determination from different carbohydrates during heat processing," *Food Chemistry*, 59 (1). 157-163. May 1997.

[31] Davies, C.G.A. and Labuza, T.P, The Maillard reaction application to confectionery products, In G. Zeigler (Ed.), Confectionery Science. Pennsylvania State University Press. 1997, 35-66.

[32] Zhang, L., Li, J. and Zhou, K, "Chelating and radical scavenging activities of soy protein hydrolysates prepared from microbial proteases and their effect on meat lipid peroxidation," *Bioresource Technology*, 101 (7). 2084-2089. Apr. 2010.

[33] Ledoux, M. and Lamy, F, "Determination of proteins and sulfobetaine with the Folin-phenol reagent," *Analytical Biochemistry*, 157 (1). 28-31. Aug. 1986.

[34] Yoshimura, Y., Iijima, T., Watanabe, T. and Nakazawa, H, "Antioxidative effect of Maillard reaction products using glucose-glycine model system," *Journal of Agricultural Food Chemistry*, 45 (10). 4106-4109. Oct. 1997.

[35] Liu, Q., Li, J., Kong, B., Li, P. and Xia, X., "Physicochemical and antioxidant properties of Maillard reaction products formed by heating whey protein isolate and reducing sugars," *International Journal of Dairy Technology*, 67 (2). 220-228. May 2014.