

# Antioxidant Activity, $\alpha$ -Glucosidase and Lipase Inhibitory Activity in Rice Miso with Kidney Bean

Chengyu Jiang<sup>1,2</sup>, Zhaohong Ci<sup>1,2</sup>, Michiyuki Kojima<sup>1,2,\*</sup>

<sup>1</sup>Department of Food Science, Obihiro University of Agriculture and Veterinary Medicine, 080-8555, 11, Nishi-2-Sen, Inada-Cho, Obihiro, Hokkaido, Japan

<sup>2</sup>Department of Bioresources Science, United Graduate School of Agricultural Sciences, Iwate University, 020-8550, 3-18-8, Ueda, Morioka, Iwate, Japan

\*Corresponding author: [kojima@obihiro.ac.jp](mailto:kojima@obihiro.ac.jp)

Received July 17, 2018; Revised August 20, 2018; Accepted August 27, 2018

**Abstract** Rice miso is a traditional Japanese seasoning food which are not only prolonging the consumption term, but also has good sensory characteristics. So thus, rice miso is very popular in Japan. In the present study, in order to improve the potential functionality, we processed rice miso adding with kidney bean (RMKB), red bean (RMRB) and buckwheat (RMBW), respectively. DPPH radical-scavenging activity, melanoidin and polyphenol content, and enzyme inhibitory activity were detected in rice miso products at different fermentation period (0, 3, 6, 24, 36 months), respectively. The results showed the DPPH radical-scavenging activity of each rice miso product was increased with prolonging the fermentation period, and RMKB showed the highest DPPH radical-scavenging activity in different rice miso products at 6, 24, 36 months. Moreover, DPPH radical-scavenging activity, melanoidin and polyphenol content of rice miso with kidney bean (RMKB) were significantly higher than those of rice miso (RM; as control) in methanol fraction purified by Diaion HP-20 column. RMKB-24M showed the highest antioxidant activity which was a better process to produce high antioxidant profiles. Furthermore, the melanoidin content in rice miso products was approximately 6-folds higher than polyphenol content. Comparing with polyphenol, melanoidin was the main antioxidant material in the rice miso products. In addition, RMKB-24M showed stronger  $\alpha$ -glucosidase and lipase inhibitions. These results suggest rice miso with kidney bean had useful components, in particular, potential applications for antioxidant, and the treatments of diabetes and anti-obesity.

**Keywords:** kidney bean, rice miso, melanoidin, DPPH radical-scavenging activity, enzyme inhibitory activity

**Cite This Article:** Chengyu Jiang, Zhaohong Ci, and Michiyuki Kojima, "Antioxidant Activity,  $\alpha$ -Glucosidase and Lipase Inhibitory Activity in Rice Miso with Kidney Bean." *Journal of Food and Nutrition Research*, vol. 6, no. 8 (2018): 504-508. doi: 10.12691/jfnr-6-8-3.

## 1. Introduction

Lifestyle diseases such as diabetes and hypertension affect the overall risk of death [1]. In recent years, lifestyle diseases have rapidly increased in the 30s and 40s, and children have also seen signs of it. Dietary life with high calorie and high fat diet not only promotes obesity but also promotes the onset of hyperlipidemia and diabetes and further causes heart disease [2]. According to an epidemiological approach, Marshall reported that humans with high fat intake had high blood insulin levels when fasting [3]. Chijimatsu also reported that blood cholesterol in rats fed a high cholesterol diet was increased [4].

Soybeans and their fermented products are famous and popular in Asian diets [5]. With the improvement of sensory characteristics and nutritious components, fermentation has become a beneficial and healthy method in food processing. As a traditional Japanese seasoning, rice miso is fermented by boiled soybean and rice-malt. It has been reported that rice miso has physiological effects as lipid

peroxidation-inhibiting action, anti-hypertension, anti-mutagen and blood glucose level elevation-inhibiting action [6,7,8].

Kidney bean is accounting for about 60% of beans in Hokkaido, Japan. It contains a large amount of dietary fiber and anthocyanin with various physiological effects such as antioxidant activity, glycolysis-inhibitory activity, Anti-obesity and Anti-cancer [9,10,11,12].

In this study, in order to development and utilization of kidney bean, we fermented Japanese traditional food rice miso with kidney bean (RMKB), and clarify the antioxidant activity and enzyme inhibitory activity with different fermentation period to compared with rice miso (RM).

## 2. Materials and Methods

### 2.1. Materials

For the experiment, we purchased kidney bean (*Phaseolus vulgaris* L.), soybean (*Glycine max*), rice-malt,

salt, seed miso from supermarket. Rice-malt was purchased from the Salt Industry Center (Japan).

Folin-Ciocalteu reagent was obtained from Nacalai Tesque, Inc. (Kyoto, Japan). DPPH (2, 2-diphenyl-1-picrylhydrazyl) was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Catechin, trolox, DNS (3, 5-dinitrosalicylic acid), and lipase were purchased from Sigma-Aldrich Co., LLC. (Tokyo, Japan). Glucose and glycine were purchased from Kanto Chemical Co., Inc. (Tokyo, Japan). Lecithin was purchased from Avanti Polar Lipids, Inc. (Alabaster, USA). The other reagents were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

## 2.2. Sample Preparation

The miso product was manufactured by a method for industrial producing rice miso. First, soybean and kidney bean of each 1.25 kg were soaked in water for 16 hours at room temperature (beans: distilled water = 1: 3 (w/w)). Then, autoclaved soaked bean (KT-3045, ALP Co., Ltd., Japan) for 20 min at 110°C. After preparing paste by crushing the steamed beans, mixed (KN1500, Taisho electric MFG. Co., Ltd., Japan) with rice-malt (2.5 kg), salt (1 kg), some seed water, and seed miso (400 g). The mixture was packed in pickle barrels (Shinkigosei Co., Ltd. Japan) and fermented at 30°C. As mentioned above, rice miso with red bean (RMRB) and rice miso with buckwheat (RMBW) were manufactured in the same way. The products were sampled for analysis at 0, 3, 6, 24, 36 months (M) after fermentation.

## 2.3. Extraction and Fractionation

Polyphenol in the miso were extracted by the method of Saito [13]. Each miso sample (5 g) were mixed with 20 mL of 80% v/v ethanol, vortexed, and ultrasonicated for 30 min. The suspension was then centrifuged at 1,006 ×g for 10 min. As mentioned above, the same operation was repeated twice. Then, mixed with 20 mL of 70% v/v acetone and the aforementioned process was repeated thrice to take the extract as mixture extract. After that, according to the Ikeda's method [14], the mixture extract was concentrated by rotary evaporation in vacuum, and constant volume to 20 mL with distilled water. Then, added the same amount of n-hexane and ethyl acetate to delaminate the solution. Subsequently, a part of water-soluble fraction was purified by chromatography through Diaion HP-20 column. The column was washed by distilled water and then eluted by methanol.

## 2.4. Melanoidin Content Determination

The content of melanoidin was determined by the method of Martins [15]. Briefly, 0.02 M of glucose and glycine were dissolved by 0.1 M phosphate buffer (pH 6.8), and heated at 120°C for 2 hours. Then, the solution was placed in a dialysis membrane (14000 MWCO, UC 36-32-100; EIDIA Corporation, Japan) and dialyzed against distilled water (7 days). The dialysate was lyophilized for 48 hours, and took as melanoidin standard. The absorbance was measured at 450 nm. The results were expressed as the mg melanoidin equivalents (ME) per gram DW miso ( $y = 63.855x + 0.4273$ ,  $R = 0.9994$ ).

## 2.5. Polyphenol Content Determination

The content of polyphenol was determined using the method of Folin-Ciocalteu [16] with slight modifications. Each sample (100 μL) was mixed with 300 μL of distilled water, 400 μL of 50% Folin-Ciocalteu reagent, and 400 μL of 10% Na<sub>2</sub>CO<sub>3</sub> aqueous solution. The reaction solution was incubated at 30°C for 30 min, and centrifuged at 1,006 ×g for 10 min. The absorbance was measured at 760 nm, and results were expressed as the mg catechin equivalents (CE) per gram DW miso ( $y = 11.834x - 0.9597$ ,  $R = 0.9969$ ).

## 2.6. DPPH Radical-scavenging Activity Assay

The DPPH radical-scavenging activity was determined by the method of Brand-Williams [11]. The extract (50 μL) was added to a microplate and mixed with 100 μL of 99.5% v/v ethanol and 150 μL DPPH solution. The solution was kept in the dark for 15 min after which its absorbance was determined at 520 nm by a microplate reader. Ten-fold diluted 2 mM trolox was used as the standard and the results were expressed as μmol trolox equivalents (TE) per gram DW miso ( $y = -13.555x + 30.842$ ,  $R = 0.9977$ ).

## 2.7. α-Glucosidase Inhibitory Activity Assay

α-Glucosidase inhibitory activity was determined by the improved DNS method [12]. A mixture of 0.5 mL of sample extract with different concentration (0.1, 0.2, 0.3 g) and 0.5 mL of the α-glucosidase solution was pre-incubated at 37°C for 10 min to prepare solution I. A mixture of 50 μL of 0.4% sucrose solution, 625 μL of 0.1M Na<sub>2</sub>PO<sub>4</sub> buffer (pH 6.8) and 125 μL of 1% NaCl was pre-incubated at 37°C for 10 min to prepare solution II. Then, 200 μL of solution I was mixed with solution II and incubated at 37°C for 30 min. The enzyme reaction was stopped by adding 125 μL of 2 N NaOH (added 2 N NaOH before incubation for blank). DNS solvent (1%, 125 μL) was added and reacted in boiling water bath for 10 min. Absorbance was measured at 540 nm. Standard curves were using glucose calibration, and expressed as α-glucosidase inhibition (%).

$$\alpha - \text{Glucosidase inhibition (\%)} = \left[ 1 - \left( \frac{A_{\text{sample}} - A_{\text{blank}}}{A_{\text{control}} - A_{\text{test}}} \right) \right] \times 100\%$$

where  $A_{\text{sample}}$  is the absorbance of the mixture of sample, sucrose solution, enzyme and DNS solvent;  $A_{\text{blank}}$  is the absorbance of the mixture of sample, sucrose solution and DNS solvent without enzyme;  $A_{\text{control}}$  is the absorbance of the mixture of buffer (instead of sample), sucrose solution, enzyme and DNS solvent;  $A_{\text{test}}$  is the absorbance of the mixture of buffer (instead of sample), sucrose solution and DNS solvent without enzyme.

## 2.8. Lipase Inhibitory Activity Assay

Lipase inhibitory activity was determined by the improved method of Han [17]. The substrate solution was prepared by adding 10 mg lecithin, 80 mg triolein, and

5 mg cholic acid to 9 mL TES buffer (pH 7.0), and sonication. Add 240  $\mu$ L of sample extracts with different concentration (0.1, 0.2, 0.3 g), 80  $\mu$ L lipase solution and 80  $\mu$ L substrate solution into a glass tube, incubate at 37°C for 30 min. Then, add 2 mL copper reagent and 4 mL chloroform, stir and centrifuge at 1,006  $\times$ g for 5 min. Transfer 2.4 mL of chloroform layer to a new glass tube and add 400  $\mu$ L of 0.1% DDTC-butanol solution and measure the absorbance at 440 nm. Standard curves were using linoleic acid, and expressed as lipase inhibition (%).

$$\text{Lipase inhibition (\%)} = \left[ 1 - \left( \frac{A_{\text{sample}}}{A_{\text{control}}} \right) \right] \times 100\%$$

where  $A_{\text{sample}}$  is the absorbance of the mixture of sample, substrate solution, enzyme and DDTC-butanol solvent;  $A_{\text{control}}$  is the absorbance of the mixture of buffer (instead of sample), substrate solution, enzyme and DDTC-butanol solvent.

## 2.9. Statistical Analysis

The experiments were repeated at least three times. Data were expressed as means  $\pm$  standard deviation. Significant differences were determined by one-way ANOVA and Fisher's test (SAS v. 7.1, SAS Institute Inc., Cary, NC, USA). Differences were considered to be significant at  $P < 0.05$ .

## 3. Results and Discussion

### 3.1. DPPH Radical-scavenging Activity in Rice Miso Products Extract

The DPPH radical-scavenging activity of four rice miso products extracts shown in Table 1. DPPH radical-scavenging activity of rice miso products was increasing with prolonging the fermentation period. Furthermore, as comparing with RM (rice miso; as control), DPPH radical-scavenging activity of RMKB, RMRB and RMBW shown significantly higher in 3 M, 6 M, 24 M and 36 M, respectively. Many studies reported the deep-colored coat of legumes had higher polyphenol content and antioxidant activity [18,19]. Therefore, we estimate that the antioxidant activity of deep-colored bean retains a high level after fermentation.

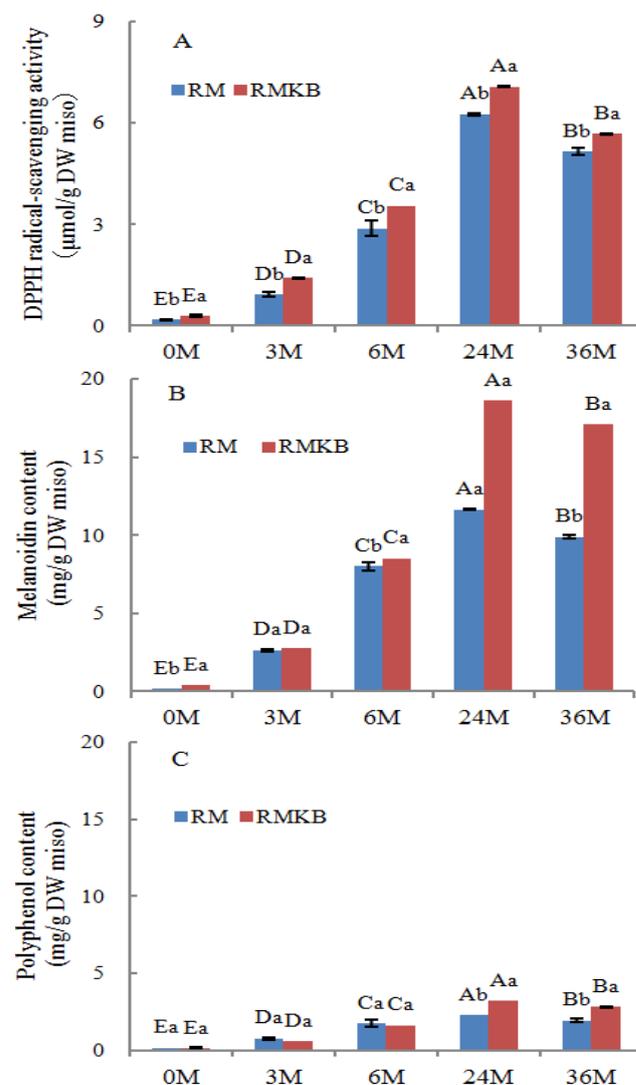
**Table 1. Changes of the DPPH Radical-scavenging Activity in 4 Types of Rice Miso Products**

Sample	DPPH radical-scavenging activity ( $\mu$ mol/g DW miso)			
	3 M	6 M	24 M	36 M
RMKB	1.3 $\pm$ 0.1Cd	2.8 $\pm$ 0.1Ac	6.2 $\pm$ 0.1Ab	6.7 $\pm$ 0.1Aa
RMRB	2.0 $\pm$ 0.1Ad	2.5 $\pm$ 0.1Bc	6.1 $\pm$ 0.2Ab	6.5 $\pm$ 0.2Ba
RMBW	1.5 $\pm$ 0.1Bd	2.4 $\pm$ 0.1Cc	5.8 $\pm$ 0.3Bb	6.4 $\pm$ 0.2Ca
RM	1.1 $\pm$ 0.1Dd	2.3 $\pm$ 0.1Dc	5.5 $\pm$ 0.1Ca	5.2 $\pm$ 0.1Db

Abbreviations: M, month; DW, dry weight; RMKB, rice miso with kidney bean; RMRB, rice miso with red bean; RMBW, rice miso with buckwheat; RM, rice miso. Data represent the mean  $\pm$  SD from at least three independent studies. Values within a column followed by different capital letters and values within a row followed by different small letters are significant at  $p < 0.05$ .

### 3.2. DPPH Radical-scavenging Activity, Melanoidin and Polyphenol Content in Methanol Fraction after HP-20 Column

The extracts from RM and RMKB were purified by HP-20 column, and methanol fraction was obtained for the following experiments. The DPPH radical-scavenging activity was significantly increasing with prolonging the fermentation period, and the highest value was in 24 months. However, comparing with RMKB-24M (7.1  $\mu$ mol/g DW miso), RMKB-36M (5.7  $\mu$ mol/g DW miso) was significantly decreased (Figure 1A). Melanoidin produced from an amino acid-sugar model system have been associated with the formation of compounds with strong antioxidant activity [20]. From 24 months, melanoidin content of miso products may be decreased with rice miso products fermentation, and resulting in DPPH radical-scavenging activity was decreased. Therefore, and rice miso product fermented at 24 months was a better process to produce high antioxidant profiles.



**Figure 1.** DPPH radical-scavenging activity, melanoidin and polyphenol content in methanol fraction after HP-20 column. Values within a same column followed by different small letters within different columns followed by different capital letters are significant at  $p < 0.05$ . A. DPPH radical-scavenging activity. B. Melanoidin content. C. Polyphenol content. Abbreviations: M, month; DW, dry weight; RMKB, rice miso with kidney bean; RM, rice miso

The melanoidin content shown in Figure 1B. The melanoidin content was increasing with prolonging the fermentation period, and the highest value was in RMKB-24M (18.6 mg/g DW miso). Moreover, we found positive relationship between melanoidin content and DPPH radical-scavenging activity (correlation coefficient;  $R = 0.9427$ ). The antioxidant activity is increasing with the prolonging the wheat miso fermentation period, and greatly involved with produced coloring component [21].

Moreover, methanol fraction after HP-20 column was analyzed polyphenol content (Figure 1C). The highest polyphenol content was in RMKB-24M (3.2 mg/g DW miso). There was also positive relationship between polyphenol content and DPPH radical-scavenging activity (correlation coefficient;  $R = 0.9570$ ). The polyphenol content of perilla miso product is increasing with DPPH radical-scavenging activity [22]. Isoflavones in fermented soybean have a strong antioxidant activity [23]. Moreover, with the prolonging fermentation period, the ratio between melanoidin and polyphenol content were from approximately 3:1 to 6:1, and melanoidin ratio was increased. Therefore, we concluded comparing with polyphenols, melanoidin was the mainly antioxidant component in rice miso products.

### 3.3. Enzyme Inhibitory Activity of RMKB

$\alpha$ -Glucosidase inhibitory activity of RMKB was shown in Table 2.  $\alpha$ -Glucosidase inhibitory activity was significantly increasing with prolonging fermentation period, and the strongest inhibition was in RMKB-24M. It has reported that other fermented soybean products have  $\alpha$ -glucosidase inhibitory activity [24]. Glucose-amino acid model MRPs showed stronger  $\alpha$ -glucosidase inhibitory activity [25].

**Table 2.  $\alpha$ -Glucosidase Inhibitory Activity of RMKB in Methanol Fraction after HP-20 Column**

Sample	$\alpha$ -Glucosidase inhibition (%)		
	0.1 g DW miso	0.2 g DW miso	0.3 g DW miso
RMKB-0M	1.5 $\pm$ 0.1Ec	5.5 $\pm$ 0.1Eb	7.0 $\pm$ 0.1Ea
RMKB-3M	4.8 $\pm$ 0.3Dc	19.2 $\pm$ 0.9Db	21.5 $\pm$ 1.1Da
RMKB-6M	22.9 $\pm$ 2.8Ac	42.7 $\pm$ 2.8Bb	50.8 $\pm$ 2.0Ba
RMKB-24M	16.2 $\pm$ 2.3Cc	55.0 $\pm$ 0.7Ab	81.2 $\pm$ 1.3Aa
RMKB-36M	21.4 $\pm$ 2.3Bc	35.4 $\pm$ 0.6Cb	38.5 $\pm$ 2.4Ca

Abbreviations: M, month; DW, dry weight; RMKB, rice miso with kidney bean. Data represent the mean  $\pm$  SD from at least three independent studies. Values within a column followed by different capital letters and values within a row followed by different small letters are significant at  $p < 0.05$ .

Lipase inhibitory activity of RMKB was shown in Table 3. Lipase inhibitory activity of RMKB was the similar trend with  $\alpha$ -glucosidase inhibitory activity. Polymeric melanoidins exhibit serum cholesterol-lowering action, intestinal lactic acid bacteria improving action [26]. Brown pigment in soybean paste (miso) have strongly anti-trypsin activity except sugar digesting enzyme activity in vivo experiments [27]. Therefore, rice miso adding with kidney bean leads to an increase in the inhibitions of  $\alpha$ -glucosidase and lipase, and the components have enzyme inhibitory activity in RMKB-24M were polyphenol and melanoidin. However, melanoidin and polyphenol are in a mixed state, we will

clarify the strength of inhibitory activity of melanoidin and polyphenol in the future.

**Table 3. Lipase Inhibitory Activity of RMKB in Methanol Fraction after HP-20 Column**

Sample	Lipase inhibition (%)		
	0.1 g DW miso	0.2 g DW miso	0.3 g DW miso
RMKB-0M	5.2 $\pm$ 0.9Db	5.7 $\pm$ 0.6Db	7.9 $\pm$ 0.3Ea
RMKB-3M	20.6 $\pm$ 1.0Bb	27.0 $\pm$ 1.7Ca	29.0 $\pm$ 2.4Da
RMKB-6M	12.2 $\pm$ 0.4Cc	31.8 $\pm$ 1.1Cb	41.7 $\pm$ 1.4Ca
RMKB-24M	33.5 $\pm$ 3.2Ab	56.5 $\pm$ 8.6Aa	67.6 $\pm$ 5.0Aa
RMKB-36M	23.2 $\pm$ 6.1Bb	44.4 $\pm$ 7.0Ba	54.9 $\pm$ 9.2Ba

Abbreviations: M, month; DW, dry weight; RMKB, rice miso with kidney bean. Data represent the mean  $\pm$  SD from at least three independent studies. Values within a column followed by different capital letters and values within a row followed by different small letters are significant at  $p < 0.05$ .

## 4. Conclusion

In the present study, we detected DPPH radical-scavenging activity, melanoidin and polyphenol content,  $\alpha$ -glucosidase inhibition and lipase inhibition in rice miso products with different fermentation periods. Comparing with RM, RMKB had significantly higher in DPPH radical-scavenging activity, melanoidin and polyphenol content, and those were increasing with prolonging the fermentation period. However, the ratio between melanoidin and polyphenol content was from 3:1 (RMKB-0M) to 6:1 (RMKB-24M), and the ratio of melanoidin content was increased with prolonging the fermentation period. Therefore, the melanoidin was the main antioxidant component rather than polyphenol in rice miso products. In addition, there were stronger  $\alpha$ -glucosidase and lipase inhibitions in RMKB-24M, and we presumed to be a synergy of polyphenol and melanoidin.

Rice miso as a traditional Japanese food, the adding with kidney bean could improve antioxidant activity and enzyme inhibitory activity. These observations also might provide important information for further research on developing health benefits of rice miso.

## Acknowledgments

We acknowledge the financial support given by the Obihiro University of Agriculture and Veterinary Medicine, as well as Iwate University, for the completion of the study.

## Statement of Competing Interests

The authors have no competing interests.

## List of Abbreviations

M: month; RM: rice miso; RMKB: rice miso with kidney bean; RMRB: rice miso with red bean; RMBW: rice miso with buckwheat; DW: dry weight; ME:

melanoidin equivalents; CE: catechin equivalents; TE: trolox equivalents; DPPH: 2, 2-diphenyl-1-picrylhydrazyl; DNS: 3, 5-dinitrosalicylic acid; DDTC: sodium diethyl-dithio- carbamic acid; MRPs: Maillard reaction products.

## References

- [1] Sundström, J., Risérus, U., Byberg, L., Zethelius, B., Lithell, H., Lind, L., "Clinical value of the metabolic syndrome for long term prediction of total and cardiovascular mortality: prospective, population based cohort study," *British Medical Journal*, 332, 878-882. 2006.
- [2] Takahashi, M., Ikemoto, S., Ezaki, O., "Effect of fat/carbohydrate ratio in the diet on obesity and oral glucose tolerance in C57BL/6J mice," *Journal of Nutritional Science and Vitaminology*, 45(5), 583-593. 1999.
- [3] Marshall, J.A., Bessesen, D.H., Hamman, R.F., "High saturated fat and low starch and fibre are associated with hyperinsulinaemia in a non-diabetic population: The San Luis Valley Diabetes Study," *Diabetologia*, 40(4), 430-438. 1997.
- [4] Chijimatsu, T., Umeki, M., Okuda, Y., Yamada, K., Oda, H., and Mochizuki, S., "The fat and protein fractions of freshwater clam (*Corbicula fluminea*) extract reduce serum cholesterol and enhance bile acid biosynthesis and sterol excretion in hypercholesterolaemic rats fed a high-cholesterol diet," *British Journal of Nutrition*, 105(4), 526-534. 2011.
- [5] Kwon, D.Y., Daily, J.W., Kim, H.J., & Park, S., "Antidiabetic effects of fermented soybean products on type 2 diabetes," *Nutrition Research*, 30(1), 1-13. 2010.
- [6] Santiago, L.A., Hiramatsu, M., and Mori, A., "Japanese soybean paste miso scavenges free radicals and inhibits lipid peroxidation," *Journal of Nutritional Science and Vitaminology*, 38(3), 297-304. 1992.
- [7] Watanabe, H., "Biological efficacies of Miso," *Journal of the Brewing Society of Japan*, 105(11), 714-723. 2010.
- [8] Ito, A., Gotoh, T., & Fujimoto, N., "Chemoprevention of cancers by miso and isoflavones," *Journal of Toxicologic Pathology*, 11(2), 79-84. 1998.
- [9] Yoshikawa, H., Kuwashima, C., Kotaru, M., "α-amylase inhibitors in the seeds of the genus *Phaseolus* and their characteristics," *Research bulletin of Kyoto Koka Women's University*, 47, 227-237. 2009.
- [10] Kojima, M., Yamashita, S., Nishi, S., Saito, Y., and Maeda, R., "Anti-oxidative effect and Liver Protective Action of Adzuki Polyphenol," *Journal of the Japanese Society for Food Science and Technology*, 53(7), 386-392. 2006.
- [11] Kojima, M., Nishi, S., Yamashita, S., Saito, Y., and Maeda, R., "Smaller Increase in Serum Cholesterol Level in Rats Fed an Ethanol Extract of Adzuki Bean Seeds," *Journal of the Japanese Society for Food Science and Technology*, 53(7), 380-385. 2006.
- [12] Saito, Y., Nishi, S., Koaze, H., Hironaka, K., and Kojima, M., "Antioxidant and Inhibitory Activity on α-Amylase and α-Glucosidase in Legume Polyphenols," *Journal of the Japanese Society for Food Science and Technology*, 54(12), 563-567. 2007.
- [13] Saito, Y., "Characterization and bio-synthesis regulation of polyphenol in legumes seed," *Journal of the Japanese Society for Food Science and Technology*, 53(7), 380-385. 2010.
- [14] Ikeda, R., Ohta, N., and Watanabe, T., "Changes of Isoflavones at Various Stages of Fermentation in Defatted Soybeans," *Journal of the Japanese Society for Food Science and Technology*, 42(5), 322-327. 1995.
- [15] Martins, S.I.F.S., Van Boeke, M.A.J.S., "Melanoidins extinction coefficient in the glucose/glycine Maillard reaction," *Food Chemistry* 83(1), 135-142. 2003.
- [16] Takahata, Y., Ohnishi-Kameyama, M., Furuta, S., Takahashi, M., and Suda, I., "Highly polymerized procyanidins in brown soybean seed coat with a high radical-scavenging activity," *Journal of Agricultural and Food Chemistry*, 49(12), 5843-5847. 2001.
- [17] Han, L.K., Gong, X.J., Kawano, S., Saito, M., Kimura, Y., Okuda, H., "Antiobesity Actions of *Zingiber officinale* Roscoe," *Journal of the Pharmaceutical Society of Japan*, 125(2), 213-217. 2005.
- [18] Oomah, B.D., Cardador-Martinez, A., and Loarca-Pina, G., "Phenolics and anti-oxidative activities in common beans (*Phaseolus vulgaris* L.)," *Journal of the Science of Food and Agriculture*, 85(6), 935-942. 2005.
- [19] Golam, M.A., Khandaker, L., Berthold, J., Gates, L., Peters, K., Delong, H. and Hossain, K., "Anthocyanin, Total Polyphenols and Antioxidant Activity of Common Bean," *American Journal of Food Technology*, 6(5), 385-394. 2011.
- [20] Kim, J.S., & Lee, Y. S., "Antioxidant activity of Maillard reaction products derived from aqueous glucose/glycine, diglycine, and triglycine model systems as a function of heating time," *Food Chemistry*, 116(1), 227-232. 2009.
- [21] Iwaya, A., Kamesawa, H., Shimono, K., Maseda, S., "Study in Physiological Function of Miso containing Barley Koji at the High ratio," *Reports of Kagoshima Prefectural Institute of Industrial Technology*, 14, 31-34. 2000.
- [22] Kashima, T., Suzuki, H., "Study on Utilization of Residue from Processing of Egoma. *Perilla frutescens* by Fermentation (II): Brewing of miso with Egoma koji," *Bulletin of the Gifu Prefectural Agricultural Technology Center*, 9, 33-35. 2015.
- [23] Hirota, A., "Polyhydroxyisoflavones from Soybean Miso and Dou-Chi," *Journal of the Brewing Society of Japan*, 101(8), 563-570. 2006.
- [24] Fujimoto(Nakagawa), T., Hosokawa, C., & Jo, M., "Inhibitory effects of traditional fermented soybean products on α-glucosidase activity," *Memoirs of the Faculty of Human Development University of Toyama*, 11(2), 83-88. 2017.
- [25] Hwang, I.G., Kim, J.Y., 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. 2011.
- [26] Miura, L., "Studies on the Physiological Functions of Food: Focus on Melanoidins and Vegetables." *The journal of Kagawa Nutrition University*, 47, 7-23. 2016.
- [27] Momose, A., Goto, N., Hayase, H., Gomyo, T., Miura, M., "Effects of Miso (Soybean Paste) on Postprandial Blood Sugar Levels," *Journal of the Japanese Society for Food Science and Technology*, 57(2), 63-69. 2010.