

# Anti-obesity Effect and Antioxidant Activity in High-fat Diet Mice Fed Fermented Buckwheat Products (Miso)

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**Abstract** Here we examined the effects of consuming fermented buckwheat products in mice fed a high-fat diet. Buckwheat was fermented with soybeans (1:1) and rice koji for 6 months (miso). The total phenolic content and 1,1-diphenyl-2-picrylhydrazyl content of the buckwheat fermentation products (miso) increased with the time of fermentation. In the in vivo experiments, mice were fed for 5 weeks with a high-fat diet with or without supplementation using 10% buckwheat miso. Dietary supplementation with buckwheat miso significantly decreased the serum aspartate transaminase level and lipid peroxidation in the liver compared with the control group. Furthermore, the mice that received buckwheat miso showed significant decreases in their body weight and retroperitoneal and mesenteric fat weights and increased fecal lipid contents compared with the control group. These results indicate that dietary supplementation with buckwheat, which is characterized by a high antioxidant activity, has health benefits such as protection of the liver and suppression of increased body weight.

**Keywords:** buckwheat, miso, high-fat diet, antioxidant, anti-obesity, total phenolic content

**Cite This Article:** Shan Wu, Shuo Feng, ZhaoHong Ci, Chengyu Jiang, Yang Cui, Yuki Sasaki, Yukina Ota, and Michiyuki Kojima, "Anti-obesity Effect and Antioxidant Activity in High-fat Diet Mice Fed Fermented Buckwheat Products (Miso)." *Journal of Food and Nutrition Research*, vol. 4, no. 6 (2016): 355-360. doi: 10.12691/jfmr-4-6-3.

## 1. Introduction

Buckwheat is a high protein food, which is widely used in noodles and sweets in Japan. In addition, it is considered to be a health food that is rich in antioxidants. In particular, rutin found in buckwheat has pharmacological effects, such as preventing vascular fragility and strengthening the vascular wall [1,2,3]. The intake of buckwheat may be associated with decreased low-density lipoprotein (LDL) cholesterol levels [4]. The ingestion of rutin and quercetin reportedly affects lipid metabolism in animals [2,5,6,7]. The two major species of buckwheat with agricultural significance are common buckwheat (*Fagopyrum esculentum* Moench) or sweet buckwheat, and Tartary buckwheat (*F. tataricum* (L.) Gaertn.) or bitter buckwheat [8]. Common buckwheat is widely cultivated in Asia, Europe, and the Americas, whereas Tartary buckwheat is mostly cultivated in Asia (e.g., China, Bhutan, Nepal, and India) with a small amount of production in the Islek region of Europe (the border regions of Luxemburg, Germany, and Belgium) [9]. In Japan, the common species is generally used to process buckwheat noodles. However, the typical use of buckwheat is mainly limited to noodles; therefore, advanced processing methods and new buckwheat products are desired.

Fermentation is a microorganism-driven process that yields high value products from raw or low grade substrates [10]. In particular, fermentation based on microorganisms is used to produce food products, and industrially produce organic compounds, i.e., starch can be broken down into smaller fragments with the release of carbon dioxide; during the brewing process, sugars can be decomposed into alcohol with the release of carbon dioxide; and in the production of bean paste (miso), proteins can be decomposed into small peptide molecules and amino acids, starch can be broken down into glucose, and fats can be decomposed into fatty acids and glycerol. Fermentation is considered to improve the nutritional value of food and break it down into more readily catabolizable forms [10,11].

Here we fermented a mixture of buckwheat and soybeans (miso), and determined the physical and chemical characteristics of the miso obtained. Furthermore, we investigated the effects of miso in terms of its antioxidant activity and antiobesity effect in mice fed a high-fat diet (HFD).

## 2. Materials and Methods

### 2.1. Miso Production [12]

The fermentation products (miso), buckwheat and soybeans (production date, 2011, expiration date, 2013.11),

were obtained from Hosokawa Seian Co. Ltd (Tokachi, Japan) and rice-koji salt was purchased from the Salt

Industry Center (Japan). The buckwheat and soybean miso (BWM) was prepared as follows (Table 1).

**Table 1. Proportions of soybeans, rice-koji, buckwheat, salt, seed water, and seed miso used in miso production**

	Steamed soybeans (kg)	Steamed buckwheat (kg)	Rice-koji (kg)	Salt (kg)	Seed water	Seed miso
BWM	2.85	3.25	2.50	1.00	400	400

Abbreviations: BWM, buckwheat-soybean miso. The water content of miso was 36% (water content = (steamed beans – dry beans + seed water)/10000 × 100).

First, 1.25 kg of raw soybeans and 1.25 kg of raw buckwheat were soaked in water (beans:distilled water = 1:3, w/w). Further, the soaked soybeans and buckwheat were placed in five 1-L beakers with the soaking water and autoclaved (KT-3045, ALP Co. Ltd, Japan) at 110°C for 20 min. The paste was prepared by crushing the steamed soybeans and buckwheat, which were mixed (KN1500, Taisho Electric MFG. Co. Ltd, Japan) with rice-koji, salt, seed water, and seed miso, as shown in Table 1. BWM was packed (10 kg) in pickle barrels (Shinkigosei Co. Ltd, Japan) and fermented at 25°C – 30°C for 180 days. The content of each container was thoroughly mixed once a month for 30 min using a mixer (KN1500). The products were sampled for analysis before fermentation (0 months) and after fermentation for 3 and 6 months. A portion of the sample obtained after fermentation for 6 months was freeze-dried and used as a dietary supplement in the animal experiments.

## 2.2. Physicochemical Properties

The samples (5 g) were extracted with 10 mL distilled water and centrifuged three times. Soluble solids were measured in water extracts using a digital pocket refractometer (PAL-Patisserie; Atago Co. Ltd) and the L-glutamic acid content was determined using an L-glutamic acid assay kit II (Yamasa Corporation Co. Ltd, Japan). The surface color of the 6 month-fermented miso was measured using a Chroma Meter (MINOLTA CR-200, Japan), and the color intensity was quantified with L\* a\* b\* scales in the CIE LAB color space [13]. The L\*, a\*, and b\* values were measured. The data were expressed as the mean based on 20 measurements.

## 2.3. Total Phenolic Content and Antioxidant Activity

Each miso sample was extracted three times with 80% ethanol and 70% acetone, where the final volume of the extract was 60 mL. Each miso extract was used to determine the total phenolic content (TPC) and antioxidant activity.

TPC was determined using the Folin–Ciocalteu method [12,14] with slight modifications. Miso extracts (100 µL) were mixed with 300 µL of distilled water, 400 µL of Folin–Ciocalteu reagent (previously diluted two-fold with distilled water), and 400 µL of Na<sub>2</sub>CO<sub>3</sub> (100 g/L) and was placed at 30°C for 30 min. The absorbance was measured at 760 nm and expressed as mg of gallic acid (1 g/L). The linearity range of the calibration curve was 0–125 µg (r = 0.998).

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay was performed according to the improved method reported by Blois [12,15]. Miso extracts (150 µL) were incubated with the same volume of 2 mM of DPPH in ethanol in the dark for 15 min, and the decrease in absorbance at 517 nm was

measured using a 96-well microplate reader (MTP300, Corona electric Co. Ltd, Japan). Standard curves were constructed using Trolox standards (0–30 nM) and the antioxidant activity of the extracts was expressed in Trolox equivalents (nM).

## 2.4. Animals and Diets

Male ddY mice (7 weeks old; Japan SLC, Inc., Shizuoka, Japan) were housed in plastic cages in an animal room, which was maintained with a 12:12 h light:dark cycle (light period, 8:00–20:00) at a constant temperature of 23°C ± 2°C and relative humidity of 60% ± 5%. Mice were randomly assigned to four groups (n = 5) and fed HFD for 2 weeks. The control group was fed HFD supplemented with 10% normal diet (HFD group), whereas the other groups were fed HFD supplemented with 10% BWM (BWM-HFD group).

The mice were allowed free access to food and water, and their intake rates were recorded. After 5 weeks, the final body weight and fecal weights were measured. On the final day of the experiment, mice were fasted overnight, anesthetized using nembutal injection (0.75 µL/g body weight), and laparotomy was performed. Blood was collected from the heart and rapidly mixed with EDTA-2Na as an anticoagulant, before separating by centrifugation at 1,000 ×g for 30 min. The livers and kidneys were collected, weighed, rapidly frozen in liquid nitrogen, and stored at –20°C until their analysis.

The experimental animals were handled according to the Guide for the Care and Use of Laboratory Animals [16] and the regulations of the National University Corporation Obihiro University of Agriculture and Veterinary Medicine for animal experiments. The experimental design was approved by the Animal Experiment Committee of Obihiro University of Agriculture and Veterinary Medicine, and it adhered to the standard principles described in the Guide for the Care and Use of Laboratory Animals [17].

## 2.5. Analysis of Sera, Organs, and Feces

Neutral lipids, free fatty acids, phospholipids, total cholesterol, LDL cholesterol, alanine aminotransaminase (ALT), aspartate transaminase (AST), and lactate dehydrogenase (LDH) levels in serum were measured using a TDX Analyzer (Abbott Japan Co. Ltd, Tokyo, Japan).

Lipid peroxidation in serum was assessed by determining the thiobarbituric acid reactive substances (TBARS), where 400 µL of 8.1% sodium dodecyl solution was added to 100 mg of liver, before adding 300 µL of 20% acetic acid buffer (pH 3.5) and 500 µL of distilled water. The mixture was homogenized using a Teflon homogenizer and 600 µL was transferred to a clean dry glass tube containing an equal volume of 25 ppm aqueous

solutions of copper (II) sulfate, and the reaction mixture was incubated for 1 h at 37°C. After the reaction, 950 µL was placed in a capped test tube, before adding 25 µL of 0.8% butylated hydroxytoluene acetic acid and 750 µL 0.8% tertiary butyl alcohol. After placing the reaction mixture for 1 h on ice, it was incubated at 100°C for 1 h in a block heater and cooled in running water, following which 500 µL of distilled water and 2.5 mL of n-butanol:pyridine (15:1) were added with vigorous stirring. The mixture was subjected to centrifugation at 1,500 ×g for 10 min, and the absorbance of the supernatant was measured at 532 nm. Lipid peroxidation was calculated as the amount of 1,1,3,3- tetraethoxypropane [18,19].

The freeze-dried fecal masses were degreased, redissolved in isopropanol, and analyzed to determine their cholesterol contents using a Wako cholesterol-E Test kit (Wako Pure Chemical Industries Ltd, Osaka, Japan).

The bile acid content was measured by extracting 100 mg of feces with three volumes of distilled water for 20 min by sonication. The extract was centrifuged at 1,000 ×g, and the supernatant was collected. The procedure was performed three times and the supernatants were combined. The bile acid content was measured using a Total Bile Acid Test Wako kit (Wako) and expressed as the amount of cholic acid [19].

## 2.6. Statistical Analyses

The data were expressed as the mean ± standard error. Significant differences were determined by ANOVA and Tukey's studentized range (Honestly Significant Difference) test (SAS 9.3, SAS Institute Inc.). Differences were considered to be significant at  $p < 0.05$ .

## 3. Results and Discussion

### 3.1. Physicochemical Properties, TPC, and Antioxidant Activity of Miso

Buckwheat fermentation (miso) includes the hydrolysis of proteins to amino acids and starch to glucose [20]. We determined the L-glutamic acid and soluble solids content of miso, which increased with the time of fermentation (Table 2).

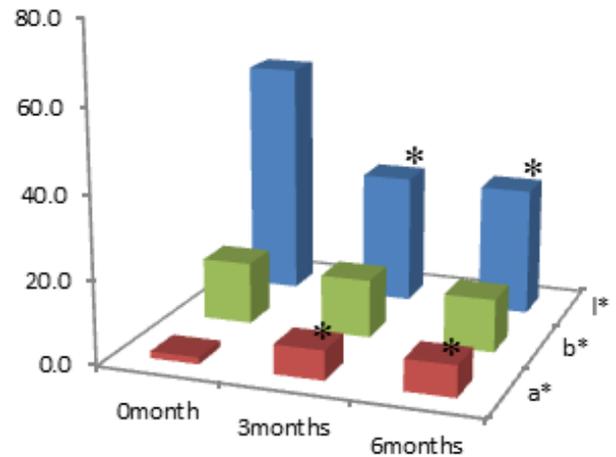
**Table 2. Changes in the L-glutamic acid and soluble solids contents in the miso produced using buckwheat and soybeans**

Fermentation time (months)	L-Glutamic acid (mg/100g miso)	Soluble solids content (Brix%)	pH
0	686±19 <sup>c</sup>	6.1±0.3 <sup>b</sup>	5.8±0.1 <sup>b</sup>
3	895±12 <sup>b</sup>	8.0±0.2 <sup>a</sup>	5.0±0.1 <sup>a</sup>
6	1011±14 <sup>a</sup>	8.5±0.4 <sup>a</sup>	4.9±0.1 <sup>a</sup>

Data are expressed as means ± SD (n = 3). Values with different superscript letters in the same column differ significantly with the fermentation time ( $p < 0.05$ ).

L\* and b\* values obtained for the samples decreased with the time of fermentation (Figure 1), whereas a\* values decreases with the time of fermentation (Figure 1).

The fermentation process used to obtain miso caused proteins to decompose into peptides and amino acids [21,22]. Thus, we suggest that the protein and starch contents of buckwheat miso were broken down into amino acids and glucose on fermentation.



**Figure 1.** Changes of the L\* (■), a\* (■) and b\* (■) in the miso mixed with buckwheat and soybeans. The superscript \* indicates significant difference in the data presented in the same internal compared with the values for 0 month ( $p < 0.05$ )

In the fermented buckwheat product, TPC and DPPH radical scavenging activity increased with the time of fermentation ( $p < 0.05$ , Table 3).

**Table 3. Changes of the total phenolic content (TPC) and diphenyl - 2 - picryl-hydrazyl (DPPH) content in the miso produced using buckwheat and soybeans**

Fermentation time (months)	TPC (mg/g DW miso)	DPPH (µmol/g DW miso)
0	1.0 ± 0.0 <sup>cc</sup>	1.1 ± 0.0 <sup>cc</sup>
3	3.9 ± 0.1 <sup>bb</sup>	4.1 ± 0.0 <sup>bb</sup>
6	5.2 ± 0.0 <sup>aa</sup>	7.9 ± 0.0 <sup>aa</sup>

Abbreviations: DW, dry weight. Data are expressed as means ± SD (n = 3). Values with different superscript letters in the same column differ significantly with the fermentation time ( $p < 0.05$ ).

The correlation between DPPH radical - scavenging activity and TPC in the fermented buckwheat product was calculated using Pearson's correlation coefficient ( $r = 0.961$ ;  $p < 0.01$ ). Takasaki et al. reported a high positive correlation between the total polyphenols content and DPPH radical-scavenging activity [ $r = 0.990$  ( $p < 0.01$ )] [23], which is consistent with the result of the present study. Therefore, we hypothesize that the antioxidant activity of buckwheat miso is closely associated with polyphenols.

### 3.2. Effects of Buckwheat Miso on Food Intake and the Body, Organ, and Fecal Weights in HFD Mice

There were no significant differences in food intake, kidney weight, and fecal weight between the groups (Table 4).

**Table 4. Food intake, body weight, fecal weight, and organ weight in HFD mice**

	HFD	BWMHF
Food intake (g/mouse/day)	5.9±0.3	6.4±0.9
Final body weight (g/mouse)	47.5±2.0	41.0±1.2*
Fecal weight (g/mouse/day)	1.0±0.1	0.9±0.1
Fecal lipids (mg/mouse/day)	61.7±5.8	83.0±8.8*
Liver weight (g/mouse)	1.9±0.1	1.6±0.1*
Kidney weight (g/mouse)	0.8±0.0	0.7±0.1

Abbreviation: HFD, high fat diet ; BWM, buckwheat and soybeans miso. Values represent means ± standard error based on five mice. \*Significant difference compared with the values for the control group,  $p < 0.05$ .

However, HFD mice fed a diet supplemented with buckwheat miso had lower final body and liver weights (13.6% and 17.5%,  $p < 0.05$ ), and increased fecal lipid contents (1.34-fold,  $p < 0.05$ ) compared with the control group (HFD). Shen et al. [24] showed that dietary supplementation with green tea polyphenols inhibited weight gain and decreased the fat mass. Gu et al. [25] reported that dietary cocoa supplementation of HFD for 10 weeks suppresses increased liver weight and ameliorates fatty liver disease symptoms in mice. Orihashi [26] showed that dietary supplementation with 1-year fermented miso for 6 weeks prevented fatty liver disease in cows. Thus, the hepatoprotective effect of miso may depend on the time of fermentation and vary according to the experimental animal species and feeding period tested. Hsu et al. [27] reported that supplementation with rutin suppressed increases in body weight, liver weight, and adipose tissue weight in HFD rats. Here we found that dietary supplementation with buckwheat miso for 5 weeks increased the excretion of lipids in feces, suppressed body weight increases, and reduced liver damage in HFD mice.

### 3.3. Effects of Buckwheat on Serum LDH, AST, and ALT, and Lipid Peroxidation in HFD Mice

Dietary supplementation with buckwheat miso reduced serum Neutral lipids, AST and ALT levels by 40.9%, 54.2% and 40.7%, respectively, compared with HFD ( $p < 0.05$ , Table 5).

**Table 5. Serum lipid contents after 5 weeks of the dietary treatment (high fat diet : miso = 9:1) in HFD mice**

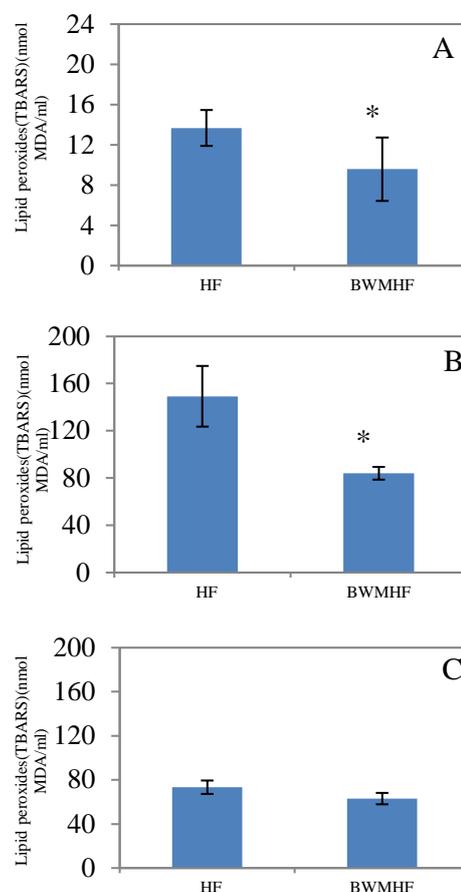
Components	HFD	BWMHF
Neutral lipids (mg/dL)	50.1±18.5	29.6±9.4*
Free fatty acid (mEq/L)	406.6±93.5	464.0±115.0
Phospholipids (mg/dL)	266.8±46.2	272.1±32.2
LDH (U/L)	458.8±132.6	557.4±105.3
AST/GOT (U/L)	289.6±64.7	132.6±26.2*
ALT/GPT (U/L)	52.6±32.9	31.2±10.0*

Abbreviation: HF, high-fat diet; BWM, buckwheat and soybeans miso; LDH, Lactate dehydrogenase; GOT, glutamic oxaloacetic transaminase; GPT, glutamic pyruvic transaminase. Values represent means ± standard deviations for five mice. \* $p < 0.05$ .

Lipid peroxidation was also decreased in the plasma and liver in the buckwheat miso groups compared with the HFD group ( $p < 0.05$ , Figure 2). Choi et al. [28] reported that rutin from Tartary buckwheat decreased A $\beta$ -induced lipid peroxidation increases in the liver and kidney.

Liver disease is characterized by elevated concentrations of liver injury markers, including ALT, AST, and c-glutamyl-transferase [29]. Here we detected decreases in the serum AST and ALT levels in the buckwheat miso-fed mice compared with the control mice. Feeding HFD rats with black soybeans and sword beans reportedly decreased the hepatic TBARS levels [30], which is consistent with our results. Gu et al. [25] reported that cocoa supplementation decreased serum ALT and hepatic triglyceride levels and suppressed liver inflammation in HFD mice. Nishi et al. observed that adzuki beans cleared the free radicals generated by inflammation and suppressed the generation of lipid peroxides, thereby suppressing inflammation of the liver [31]. Therefore, the beneficial effects of buckwheat miso supplementation in

vivo are attributable to its antioxidant activity (due to the polyphenol content of buckwheat miso), which eliminate the free radicals generated by inflammation and suppress hepatic lipid peroxidation, thereby protecting the liver against HFD.

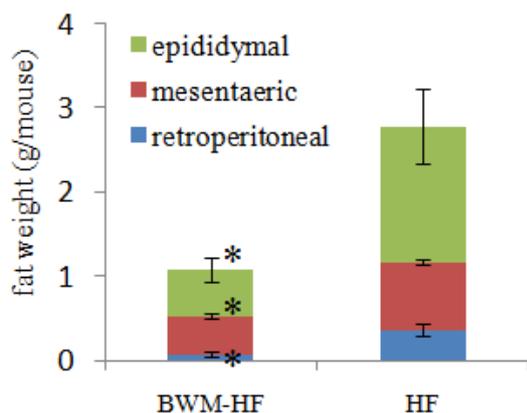


**Figure 2.** Lipid peroxides of plasma(A), liver(B), kidney(C) after 5 weeks of dietary treatment (high fat diet : miso = 9 : 1) in mice. MDA, malondialdehyde; TBARS, Thiobarbituric acid reactive substances. The superscript \* indicates significant difference in the data presented in the seam internal compared with the values for control ( $p < 0.05$ )

### 3.4. Effects of buckwheat miso on serum lipids and adipose tissue weight in HFD mice

We analyzed the serum lipids, including free fatty acids and phospholipids; however, no significant difference was observed with regard to free fatty acids and phospholipids between the two groups (Table 5). However, in HFD mice supplemented with buckwheat miso, the neutral lipids decreased (40.8%,  $p < 0.05$ ) compared with the control group (HFD). Tomotake et al. [32] showed that buckwheat protein extract decreased the triacylglycerol contents in the serum of rats compared with a control group. Qu et al. [33] showed that the consumption of noodles with added rutin decreased the free fatty acid contents in the serum of rats. reportedly, buckwheat is rich in rutin, which has an anti-obesity effect. In our study, supplementation with buckwheat miso for 5 weeks affected the neutral lipids content in the serum of HFD mice.

The retroperitoneal, mesenteric, and epididymal fat weights were lower in HFD mice supplemented with buckwheat miso ( $p < 0.05$ ) than in the control group (HFD, Figure 3).



**Figure 3.** Fat weight of retroperitoneal (■), mesenteric (■), and epididymal (■) in mice after 5 weeks of dietary treatment (high fat diet : miso = 9 : 1). HF, high -fed added 10% normal diet; BWM-HF, high -fed added 10% buckwheat miso; The superscript \* indicates significant difference compared with the values for HF ( $p < 0.05$ ).

White adipose tissue is the primary site for energy storage as triacylglycerols under conditions of excess energy [34]. Shen et al. [24] showed that supplementation with green tea polyphenols decreased the fat mass and increased the fat-free mass. Thus, we suggest that supplementation with buckwheat miso reduced the visceral fat weight in HFD mice due to the polyphenols present in the buckwheat miso.

## 4. Conclusion

In this study, we showed that dietary supplementation with buckwheat miso decreased the liver weight, serum AST and ALT levels, lipid peroxidation in the liver and serum, body weight, serum neutral lipids, and adipose tissue weight and increased fecal lipid contents in HFD mice. Our results indicate that buckwheat miso had a protective effect on the liver, an antioxidant effect, and it inhibited weight gain in the HFD ddY strain mice. Therefore, buckwheat miso may have an anti-obesity effect.

## References

- [1] Grassi, D., Desideri, G. and Ferri, C., Flavonoids: Antioxidants against atherosclerosis. *Nutrients*, 2 (8). 889-902. 2010.
- [2] Choi, I., Park, Y., Choi, H. and Lee, E.H. Anti-adipogenic activity of rutin in 3T3-L1 cells and mice fed with high-fat diet. *BioFactors*, 26 (4). 273-281. 2006.
- [3] Rivera, L., Morón, R., Sánchez, M., Zarzuelo, A. and Galisteo, M. Quercetin ameliorates metabolic syndrome and improves the inflammatory status in obese Zucker rats. *Obesity*, 16 (9). 2081-2087. 2008.
- [4] Choy, A.L., Morrison, P.D., Hughes, J.G., Marriott, P.J. and Small, D.M. Quality and antioxidant properties of instant noodles enhanced with common buckwheat flour. *Journal of Cereal Science*, 57 (3). 281-287. 2013.
- [5] Panchal, S.K., Poudyal, H., Arumugam, T.V. and Brown, L. Rutin attenuates metabolic changes, nonalcoholic steatohepatitis, and cardiovascular remodeling in high-carbohydrate, high-fat diet-fed rats. *The Journal of Nutrition*, 141 (6). 1062-1069. 2011.
- [6] Hsu, C.L., Wu, C.H., Huang, S.L. and Yen, G.C. Phenolic compounds rutin and o-coumaric acid ameliorate obesity induced by high-fat diet in rats. *The Journal of Agriculture and Food Chemistry*, 57 (2). 425-431. 2009.
- [7] Kanashiro, A., Andrade, D.C.O., Kabeya, L.M., Turato, W.M., Faccioli, L.H., Uyemura, S.A. and Lucisano-Valim, Y.M. Modulatory effects of rutin on biochemical and hematological parameters in hyper cholesterolemic Golden Syrian hamsters. *Anais da Academia Brasileira de Ciências*, 81 (1). 67-72. 2009.
- [8] Zhu, F. Chemical composition and health effects of Tartary buckwheat. *Food Chemistry*, 203. 231-245. 2016.
- [9] FAOSTAT (Statistics division of food and agriculture organization of the united nations). (2016). Available from <http://faostat3.fao.org/browse/Q/QC/E> Accessed 4.02.16.
- [10] Hussain, A., Bose, S., Wang, J.H., Yadav, M.K., Mahajan, G.B., and Kim, H. Fermentation, a feasible strategy for enhancing bioactivity of herbal medicines. *Food Research International*, 81. 1-16. 2016.
- [11] Parvez, S., Malik, K., Ah Kang, S., and Kim, H. Y. Probiotics and their fermented food products are beneficial for health. *Journal of Applied Microbiology*, 100 (6). 1171-1185. 2006.
- [12] Wu, S., Feng, S., Ci, Z., Jiang, C., Cui, Y., Sasaki, Y., Ota, Y. and Kojima, M. Fermented miso with adzuki beans or black soybeans decreases lipid peroxidation and serum cholesterol in mice fed a high-fat diet. *Journal of Food and Nutrition Research*, 3. 131-137. 2015.
- [13] CIE, Colorimetry (Second Edition), Central Bureau of the Commission International de L'Eclairage, Viena, 1986.
- [14] Rakić, S., Petrović, S., Kukić, J., Jadrnin, M., Tesević, 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. 830-834. 2007.
- [15] Musa, K.H., Abdullah, A. and Al-Haiqi, A. Determination of DPPH free radical scavenging activity: Application of artificial neural networks. *Food Chemistry* 194. 705-711. 2016.
- [16] Guide for the care and use of laboratory animals. Institute of laboratory animal resources commission on life sciences, National Academy Press, Washington, D.C. 1996.
- [17] National Research Council, Guide for the Care and Use of Laboratory Animals. National Academy Press, Washington, 1996.
- [18] Wen, B., Gao, Q.F., Dong, S.L., Hou, Y.R., Yu, H.B. and Li, W.D. Effects of different feed ingredients on growth, fatty acid profiles, lipid peroxidation and aminotransferases activities of sea cucumber *Apostichopus japonicus* (Selenka). *Aquaculture*, 454. 176-183. 2016.
- [19] Kojima, M., Yamashita, S., Nishi, S., Saito, Y., Maeda, R. Antioxidative effect and liver protective action of Adzuki polyphenol. *Nippon Shokuhin Kagaku Kogaku Kaishi*, 53. 386-392. 2006.
- [20] Wijngaard, H.H. and Arendt, E.K. Buckwheat. *Cereal Chem.*, 83. 391-401. 2006.
- [21] Kobayashi, M. Immunological functions of soy sauce: Hypoallergenicity and antiallergic activity of soy sauce. *Journal of Bioscience and Bioengineering*, 100. 144-151. 2005.
- [22] Frias, J., Song, Y.S., Martinez-Villaluenga, C., Mejia, E.G.D. and Vidal-Valverde, C. Immunoreactivity and amino acid content of fermented soybean products. *Journal of Agricultural and Food Chemistry*, 56, 99-105, 2008.
- [23] Takasaki, S., Kouda, I., Ishikawa, M., Okamoto-kainuma, A., Koizumi, Y. and Fukuda, Y. Properties of the novel products of miso developed using sesame flour. *Journal of Brewing Society of Japan*, 105. 749-758. 2010 (in Japanese).
- [24] Shen, C.L., Cao, J.J., Dagda, R.Y., Chanjaplammoovil, S., Lu, C., Chyu, M.C., Gao, W., Wang, J.S. and Yeh, J.K. Green tea polyphenols benefits body composition and improves bone quality in long-term high-fat diet-induced obese rats. *Nutrition Research*, 32 (6). 448-457. 2012.
- [25] Gu, Y., Yu, S. and Lambert, J.D. Dietary cocoa ameliorates obesity-related inflammation in high fat-fed mice. *European Journal of Nutrition*, 53 (1). 149-158. 2014.
- [26] Orihashi, T. Research about effect of miso on prevention of fatty liver in cow. *Journal of Brewing Society of Japan*, 107. 19-24. 2012.
- [27] Hsu, C.L., Wu, C.H., Huang, S.L. and Yen, G.C. Phenolic compounds rutin and o-coumaric acid ameliorate obesity induced by high-fat diet in rats. *Journal of Agriculture and Food Chemistry*, 57 (2). 425-431. 2009.
- [28] Choi, J.Y., Cho, E.J., Lee, J.M., Lee, D.G., Cho, S., Lee, S., Lee, J.M. and Yoon, Y.H. The n-butanol fraction and rutin from tartary buckwheat improve cognition and memory in an in vivo model of amyloid- $\beta$ -induced Alzheimer's disease. *Journal of Medicinal Food*, 18 (6). 631-641. 2015.
- [29] Clark, J., Brancati, F. and Diehl, A. The prevalence and etiology of elevated aminotransferase levels in the United States. *American Journal of Gastroenterology*, 98 (5). 960-967. 2003.

- [30] Byun, J.S., Han, Y.S. and Lee, S.S. The effects of yellow soybean, black soybean, and sword bean on lipid levels and oxidative stress in ovariectomized rats. *International Journal for Vitamin and Nutrition Research*, 80 (2). 97-106. 2013.
- [31] Nishi, S., Yamashita, S., Saito, Y., Maeda, R. and Kojima, M. Smaller increase in serum cholesterol level in rats fed an ethanol extract of adzuki bean seeds. *Nippon Shokuhin Kagaku Kogaku Kaishi*, 53. 380-385. 2006 (in Japanese).
- [32] Tomotake, H., Yamamoto, N., Yanaka, N., Ohinata, H., Yamazaki, R., Kayashita, J. and Kato, N. High protein buckwheat flour suppresses hypercholesterolemia in rats and gallstone formation in mice by hypercholesterolemic diet and body fat in rats because of its low protein digestibility *Nutrition*, 22. 166-173. 2006.
- [33] Qu, Y., Yasuda, T., Nakajima, K., Hiwatashi, A., Moroi, C., Sanada, H. and Egashira, Y. Effect of rutin in buckwheat noodle on lipid metabolism in rats. *Food Science and Technology Research*, 19 (6). 1011-1018. 2013.
- [34] Jung, S., Lee, M.S., Shin, Y., Kim, C.T., Kim, I.H., Kim, Y.S. and Kim, Y. Anti-obesity and anti-inflammatory effects of high hydrostatic pressure extracts of ginseng in high-fat diet induced obese rats. *Journal of Functional Foods*, 10. 169-177. 2014.