Prev. Nutr. Food Sci. 2019;24(2):144-149
https://doi.org/10.3746/pnf.2019.24.2.144
pISSN 2287-1098 · eISSN 2287-8602

Effects of 'Superhongmi' Rice Bran Extracts on Biochemical Markers of Glycolysis and Bone Metabolism in Ovariectomized Rats

Soo Im Chung[1,2], Young In Kwon[3], and Mi Young Kang[2]

[1]International Agricultural Training Center and [2]Department of Food Science and Nutrition, Brain Korea 21 Plus, Kyungpook National University, Daegu 41566, Korea
[3]Department of Food and Nutrition, Hannam University, Daejeon 34430, Korea

ABSTRACT: Middle aged women naturally enter menopause, which increases the risk of several metabolic diseases. The objective of this research was to investigate the regulatory effects of bioactive natural product "Superhongmi" rice bran on hyperglycemia, oxidative stress, and bone metabolism. The ovariectomized Sprague-Dawley rats were randomly divided into three dietary groups (n=10): ovariectomized (OVX)-AIN93M diet (OVX-AIN93M) and OVX-AIN93M diet supplemented with either low dose Superhongmi extract (1 g/kg, OVX-LSH) or high dose Superhongmi extract (5 g/kg, OVX-HSH). Body weight, activities of glucose regulatory, antioxidant enzymes, and bone metabolism biochemical markers of rats were measured. After eight weeks of feeding, the OVX-AIN93M group showed a significant increase in body weight gain relative to the sham-operated group. Superhongmi extract diet supplementation (OVX-HSH group) significantly suppressed hyperglycemia, oxidative stress, and bone resorption. These findings indicated that OVX-HSH has a potential therapeutic effect on menopause women.

Keywords: antioxidant, functional rice, menopause, rice bran, Superhongmi

INTRODUCTION

Since the 1990s, Korea has developed and distributed various types of highly functional rice to enhance value of rice. For processing purposes, a variety of aromatic rice, such as Hyangmibyeo, Hyangnambyeo, Sulhyangchalbyeo, and Aranghyangchalbyeo, were developed (Ha et al., 1996; Ha et al., 2003; Lim et al., 1998; Moon et al., 1998a; Moon et al., 2003). The varieties of Heukginjubyeo, Superjamibyeo, and Keunnunjamibyeo (Moon et al., 1998b; Yang et al., 2011; Kwon et al., 2011; Ham et al., 2015; Park et al., 2000; Ryu et al., 2006) have been developed for promoting health benefits due to antioxidant properties resulting from high levels of anthocyanin and tannin compounds. Phenolic complexes, including anthocyanin or tannin, are widely distributed in the plant world. These complexes are one of the secondary metabolites of plants that, along with various molecular structures, has physiological functions such as antioxidant, anti-cancer, and anti-bacterial activity (Wang et al., 1997). Thus, rice that has been bred specifically for its health benefits is that of the colored rice family.

In this study, we investigated the new reddish-brown rice variety "Superhongmi" as a functional material and health food. Superhongmi rice varieties typically have lower crude oil and protein contents than other colored rice varieties, and higher levels of oleic and linoleic acids. These varieties contain high amounts of the essential amino acids tyrosine and arginine, and have total polyphenols contents of 248 mg/100 g, six times higher than those of other pigmented rice varieties (Ryu, 2015). We selected these strains for their high taxifolin contents, which has anti-diabetic effects and which may be used for development of healthy and functional foods to improve post-menopausal glycolysis and bone metabolism. After mass production, Superhongmi rice endosperm is consumed daily, often steamed. The rest of the Superhongmi rice bran may potentially be used as a functional health food. Using both the endosperm and bran of rice increases the value of the rice since it can be used as two types of food.

As the average age of the population increases, it is very

Received 10 January 2019; Accepted 12 April 2019; Published online 30 June 2019

Correspondence to Mi Young Kang, Tel: +82-53-950-6235, E-mail: mykang@knu.ac.kr
Author information: Soo Im Chung (Researcher), Young In Kwon (Professor), Mi Young Kang (Professor)

important to relieve the symptoms of menopause in women, which decreases quality of life and is associated with health problems (Newton et al., 2005; Osmers et al., 2005). Physical symptoms include osteoporosis, during which bone strength decreases as a result of decreased levels of female hormones. Although many hormonal treatments are available to alleviate these symptoms, increases in breast cancer is associated with duration of hormone therapy (Kronenberg and Fugh-Berman, 2002). Therefore, there is a growing interest in diets that contain functional materials that may help alleviate menopause symptoms. Consequntly, healthy diets that can help alleviate the symptoms of menopause have been recently developed. In Korea, pomegranate extract, compound extracted from *Cynanchum wilfordii* Hemsley and *Sophora japonica* extracts have been developed into functional foods. These functional health foods show rising profits due to growing sales, indicating that these foods are very marketable (Zhang et al., 2017).

Superhongmi bran extracts also contain larger amounts of taxifolin, which is known for its anti-diabetic effects, compared to ordinary rice (Ryu, 2018). In this study, we used removed the ovaries of rats to determine the anti-diabetic effects of bran extracts and the ability of the extracts to improve glycolysis and bone metabolism potency after menopause. We suggest that Superhongmi bran by-products may be used in healthy, functional food that could improve glycolysis and bone metabolism in women after menopause.

MATERIALS AND METHODS

Materials

Superhongmi bran was from the Department of Agricultural Science, Korea National Open University, Seoul, Korea, and rice bran extracts was provided by Erom (Seongnam, Korea). Briefly, 800 g of Superhongmi bran was mixed with 50% ethyl alcohol (1:10, w/v) and shaked in an incubator (WSB-45, Daihan Scientific Co., Ltd., Wonju, Korea) at 150 rpm for 16 h. Bran extracts were filtered through Whatman filter paper (No. 2, GE Healthcare, Buckinghamshire, UK), and centrifuged at 7,000 *g* for 1 h. The extract portion was vacuum-evaporated (N-1110, EYELA, Tokyo, Japan) at 60°C, freeze-dried and kept at −20°C until analysis.

Determination of taxifolin content

50% EtOH extract filtered with a 0.2 μm syringe filter was analyzed using high-performance liquid chromatography (Tosoh 8010 Series, Tosoh Corporation, Tokyo, Japan) equipped with a TSKgel-ODS 80 (15 cm×7.6 mm, TSK ODS 80, Tosoh Corporation) column and a UV/VIS detector (TSK ODS 80, Tosoh Corporation). The mobile solvent buffer A was 0.05% phosphoric acid distilled water and solvent buffer B was 60% methanol. The flow rate was 1.0 mL/min and the injection quantity was 20 μL. Measurements were recorded at 295 nm (Goufo and Trindade, 2014).

Experimental animals and diet

Thirty 12-week-old ovariectomized (OVX) rats and 10～12-week-old sham-operated rats weighing 220～230 g were used in this study (Central Laboratory Animal Inc., Seoul, Korea). All animals were maintained in a temperature-controlled room (24±2°C, 50% relative humidity) with a 12/12 h light-dark cycle and *ad libitum* food and water. After 1 week for adaptation, rats were separated into 4 groups (n=10): SHAM, sham operated+AIN93M; OVX-AIN93M, ovariectomized+AIN93M; OVX-LSH, ovariectomized+AIN93M+low dose Superhongmi (1 g/kg body weight); OVX-HSH, ovariectomized+AIN93M +high dose Superhongmi (5 g/kg body weight). Body weights were determined every week during the feeding period (8 weeks). All animal procedures were approved by the Committee of the Kyungpook National University (approval no. 2016-0116).

Sample preparation for plasma and tissue enzyme source

After fasting for 12 h animals were sacrificed through injection of ketamin-HCl and anesthesia into muscle. Blood was extracted from the veins below the abdomen (inferior vena cava). After treating with heparin, samples were centrifuged for 15 min at 1,000 *g*, 4°C, and the plasma was collected and stored at 70°C until analysis. Liver and kidney tissues were mixed with 0.25 M sucrose solution and placed on ice for homogenization. Samples were centrifuged for 10 min at the 600 rpm to obtain the supernatant. Samples were centrifuged for a second time for 20 min at 12,000 rpm to obtain the mitochondrial fraction. The supernatant was centrifuged again for one hour at 10,500 rpm to separate into cytosolic and microsomal fractions (Hulcher and Oleson, 1973).

Measurement of enzyme activity for glucose metabolism

Glucose-6-phosphatase (G6P) activity was spectrophotometrically measured (Alegre et al., 1988). The reaction mixture contained 100 μL 18 mM ethylenediaminetetraacetic acid (EDTA, pH 6.5), 100 μL 265 mM glucose-6-phosphate (pH 6.5), 10 μL 0.2 M nicotinamide adenine dinucleotide phosphate, and 765 μL 131.6 mM 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES, pH 6.5); mutarotase and glucose dehydrogenase were then added at concentrations of 0.6 IU/mL and 6 IU/mL, respectively. After incubating samples in the reaction mixture at 37°C for 4 min, the absorbance was measured at 340 nm [nmol nicotinamide adenine dinucleotide phosphate hydrate (NADPH) oxidizaed/min/mg protein]. Glu-

cokinase (GK) activity was analysed following the method by Davidson and Arion (1987) with modifications. 100 μL KCl (1 M), 100 μL dithio erythritol (25 mM), 100 μL $MgCl_2$ (75mM), 5 μL bovine serum albumin (10 mg/mL), 10 μL of nicotinamide adenine dinucleotide (50 mM), 10 μL glucose-6-phosphate dehydrogenase (1,000 unit), 10 μL glucose (1 M), and 600 μL HEPES-NaOH (pH 7.4, 83.33 mM) was added to 10 μL of cytoplasm, and reaction was incubated at 10 min at 37°C. Finally, 10 μL adenosine triphosphate (50 mM) was added to the reaction and the change in absorbance was measured at 340 nm for 10 min. Activity was expressed as nmol of nicotinamide adenine dinucleotide hydrate (NADH) generated per min per mg of cytosolic protein (nmol NADH oxidized/min/mg protein).

Measurement of enzyme activity for antioxidant metabolism

Measurement of glutathione peroxidase (GPx) activity was based on the method by Paglia and Valentine (1967). The amount of NADPH [and glutathione disulfide (GSSG)] absorption decreases from 340 nm when oxidized glutathione (GSH) is reduced by glutathion reductase (GR) and NADPH. 2.6 mL Tris-HCl (pH 7.2, 0.1 M) buffer, 0.1 mL GSH solution (30 mM), and 6.25 μM H_2O_2 were added to 0.1 mL NADH solution (6 mM) and incubated at 25°C for 5 min; 0.1 mL cytoplasm fraction (0.4～0.6 mg protein) was then added and the reaction was incubated at 25°C for 5 min, and the change in absorbance was measured at 340 nm. Enzyme activity was expressed as the amount of enzyme used to detect 1 nmol oxidative NADPH per min. Catalase (CAT) activity was measured the method of Aebi (1974) with modifications. 2.89 mL potassium phosphate buffer (KH_2PO_4 : K_2HPO_4, pH 7.4, 50 mM) was mixed with 10 μL of enzyme source mitochondria, incubated for 5 min at 25°C, and added to 0.1 mL of a 340 mL H_2O_2 solution. The change in absorbance was measured at 240 nm (Beckman 650 spectrophotometer, Beckman Coulter Inc., Brea, CA, USA) for 5 min at 25°C. Enzyme activity was expressed as μmole of H_2O_2 broken down by 1 mg mitochondrial protein per min (μmole H_2O_2 decreased/min/mg mitochondrial protein). GR activity is a measure of oxidized NADPH since it reduces GSSG to GSH. A reaction mixture of 0.1 M potassium phosphate buffer (pH 7.4), 1 mM EDTA, 1 mM GSSG, and 0.1 mM NADPH was made to a total volume of 1 mL. Ten microliters of the enzyme cytosolic fraction was then added and the reduction in absorbance at 340 nm was measured at 25°C for two min. The activity unit was expressed as nmol of oxidized NADPH per min per mg of cytosolic protein (Mize and Langdon, 1962). Paraoxonase (PON) activity was measured using the method of Mackness et al. (1991) with a slight modification.

Measurement of bone metabolism biochemical markers

N-terminal telopeptide type 1 collagen (NTX-1), N-terminal telopeptide type 1 collagen (CTX-1), 17-β-estradiol, intact parathyroid hormone, and osteocalcin were analyzed using commercial assay kits (MyBiosource Inc., San Diego, CA, USA).

Statistical analysis

Data were analyzed by one-way analysis of variance (ANOVA) to test the effects of the experimental diets. All statistical analyses were performed using SPSS (SPSS Inc., Chicago, IL, USA). *P*-values <0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Taxifolin content

Measurements of taxifolin content, an indicator of Superhongmi bran extracts, are shown in Table 1. Taxifolin is a type of flavonoid compound, recognized as a physiological active ingredient due to effects such as strong antioxidant activity, anti-diabetes, and inhibition of blood glucose (Ryu, 2018; Kim et al., 2015). Superhongmi bran extracts contain 58.85 mg taxifolin per 100 g dried weight,

Table 1. Taxifolin contents in Superhongmi extracts (unit: mg/100 g dried weight)

	Taxifolin
Superhongmi	58.85±1.06
Brown rice	ND

Values are mean±SD (n=3).
ND, not detected.

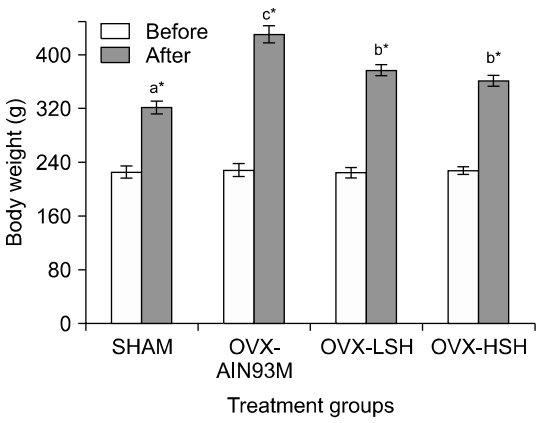

Fig. 1. Effects of Superhongmi extract on changes in body weight gain. The data are presented as mean±SE. Means not sharing a common letter (a-c) are significantly different at $P<0.05$, and asterisk indicates significant difference ($P<0.05$) between before and after weight supplement intake. SHAM, sham operated+AIN93M; OVX-AIN93M, ovariectomized+AIN93M; OVX-LSH, ovariectomized+AIN93M+low dose Superhongmi; OVX-HSH, ovariectomized+AIN93M+high dose Superhongmi.

Table 2. Effects of glucose regulating enzyme activity in ovariectomized (OVX) rats fed the normal diet and the Superhongmi extract diet

	SHAM	OVX-AIN93M	OVX-LSH	OVX-HSH
Hepatic enzyme activity (nmol/min/mg protein)				
Glucose-6-phosphatase	30.85±0.88[a]	63.00±0.39[c]	53.98±1.70[b]	49.47±1.61[b]
Glucokinase	19.28±0.12[c]	1.43±0.05[a]	2.20±0.20[a]	3.89±0.42[b]
Kidney tissue enzyme activity (μmol/min/mg protein)				
Glucose-6-phosphatase	40.02±1.47[a]	86.56±2.23[c]	67.01±2.58[b]	66.61±1.46[b]
Glucokinase	8.55±0.12[d]	2.25±0.09[a]	5.44±0.17[b]	6.38±0.23[c]

Values are mean±SE (n=10).
Means in the same row not sharing a common letter (a-d) are significantly different at $P<0.05$.
SHAM, sham operated+AIN93M; OVX-AIN93M, ovariectomized+AIN93M; OVX-LSH, ovariectomized+AIN93M+low dose Superhongmi; OVX-HSH, ovariectomized+AIN93M+high dose Superhongmi.

however taxifolin is not detected in brown rice extracts. According to a study by Ryu (2018), taxifolin, which has known anti-diabetic effects, only exists as Superhongmi varieties; other brown-colored varieties, such as Jeokjinju, Hongjinju, and Keonganghongmi, do not contain taxifolin.

Body weight gain

Changes in body weight were measured for 8 weeks (Fig. 1). No significant changes in body weight in any rat groups were observed in the first few weeks. At the end of the experiment, rats in the OVX-AIN93M group showed a marked increase in the body weight ($P<0.05$). Depending on the intake dose, OVX-HSH group and OVX-LSH group increased by approximately 135 g and 155 g, respectively, corresponding with significant weight loss in the OVX-HSH group ($P<0.05$).

Enzyme activities of glucose regulation

G6P is an enzyme involved in glycogenesis. For the OVX groups, liver and kidney G6P activities were significantly lower for rats fed the Superhongmi diet compared with the normal diet group. This suggests that Superhongmi bran extracts may have positive effects on glucose levels in those with various menopausal disorders. GK is responsible for increasing insulin secretion in pancreatic β-cells (Zhao et al., 2018). There was no significant difference in GK in the liver or kidney of rats from the OVX groups receiving either OVX-AIN93M group or OVX-LSH group. However, the OVX-HSH group significantly higher amount of GK activity of approximately 1.76-fold was observed in the liver (Table 2). Taxifolin reduces levels of blood glucose, urea nitrogen, creatinine, and expression of caveolin-1/nuclear factor-κB in streptozotocin (STZ)-introduced diabetic nephropathy rats (Matschinsky, 1990). The hypoglycemic effect is probably due to high contents of phenolic compounds and fiber (Rukmini, 2000). Lipoate activates α-lipoic acid in rice bran, which decreases glucose levels and accelerates secretion of uncoupling protein-1; lipoate was therefore used as a therapeutic agent against diabetes and obesity (Qureshi et al., 2002). From these results, we suggest that Superhongmi may lead to a decrease in G6P activity and an increase in GK activity.

Table 3. Effects of antioxidant enzyme activity in OVX rats fed the normal diet and the Superhongmi extract diet

	SHAM	OVX-AIN93M	OVX-LSH	OVX-HSH
Hepatic enzymes (nmol/min/mg protein)				
GPx	2.39±0.13[b]	1.45±0.03[a]	2.13±0.08[b]	2.32±0.02[b]
CAT	2.12±0.08[b]	1.48±0.07[a]	1.88±0.10[ab]	1.48±0.22[a]
GR	11.25±0.50[c]	6.60±0.25[a]	9.36±0.30[b]	7.89±0.27[ab]
PON	0.23±0.01[c]	0.02±0.00[a]	0.13±0.02[b]	0.17±0.01[b]
Kidney tissue enzyme activity (μmol/min/mg protein)				
GPx	2.03±0.09[c]	1.55±0.12[a]	1.66±0.05[ab]	1.76±0.06[bc]
CAT	1.32±0.10[b]	0.82±0.01[a]	0.82±0.02[a]	0.86±0.01[a]
GR	21.45±0.63[b]	16.14±0.15[a]	16.20±0.27[ab]	17.66±0.16[ab]

Values are mean±SE (n=10).
Means in the same row not sharing a common letter (a-c) are significantly different at $P<0.05$.
SHAM, sham operated+AIN93M; OVX-AIN93M, ovariectomized+AIN93M; OVX-LSH, ovariectomized+AIN93M+low dose Superhongmi; OVX-HSH, ovariectomized+AIN93M+high dose Superhongmi; SOD, superoxide dismutase; CAT, catalase; GPx, glutathione peroxidase; GR, glutathione reductase; PON, paraoxonase.

Enzyme activities of antioxidant metabolism

Reactive oxygen species (ROS) are produced when the body is under oxidative stress. When ROS accumulates, unsaturated fatty acids and lipids cause oxidative reactions; this is known to cause cardiovascular diseases and diabetes (Qureshi et al., 2002). The antioxidant enzymes superoxide dismutase, CAT, GPx, GR, and PON remove free radicals and H_2O_2 to prevent further tissues damage. Increased activities of these enzymes may play beneficial roles in improving human health (Aruoma, 1998; Halliwell, 1994). In this present study, OVX-LSH and OVX-HSH of GPx activity were 1.47 and 1.6 times higher than OVX-AIN93M, respectively (Table 3). GR activity was 6.60 nmol/min/mg protein in the OVX-AIN93M group, 9.36 nmol/min/mg protein in the OVX-LSH group, and 7.89 nmol/min/mg protein in the OVX-HSH group; we therefore showed that rats fed Superhongmi extracts

Table 4. Effect of plasma bone mineral density in OVX rats fed the normal diet and the Superhongmi extract diet

	SHAM	OVX-AIN93	OVX-LSH	OVX-HSH
CTX-1 (μg/mL)	1.53±0.11[a]	5.66±0.24[b]	2.54±0.15[c]	1.67±0.17[a]
NTX-1 (nmol/L)	100.33±3.33[a]	247.00±10.00[b]	230.33±8.82[b]	127.00±5.77[a]
Osteocalcin (ng/mL)	11.59±0.90[b]	6.48±0.02[a]	6.97±0.39[a]	6.12±0.75[a]
17-β-estradiol (ng/mL)	2.62±0.09[c]	1.81±0.04[a]	2.30±0.03[b]	2.48±0.01[bc]
Parathormone (pg/mL)	29.83±0.50[b]	24.94±0.44[a]	25.94±0.81[a]	25.82±0.19[a]

Values are mean±SE (n=10).
Means in the same row not sharing a common letter (a-c) are significantly different at $P<0.05$.
SHAM, sham operated+AIN93M; OVX-AIN93M, ovariectomized+AIN93M; OVX-LSH, ovariectomized+AIN93M+low dose Superhongmi; OVX-HSH, ovariectomized+AIN93M+high dose Superhongmi; CTX-1, C-terminal telopeptide type 1 collagen; NTX-1, N-terminal telopeptide type 1 collagen.

had higher GR activity than those fed the general diet. The OVX-LSH and -HSH groups showed 6.5-fold and 8.5-fold higher concentrations of PON than the OVX-AIN93M group, respectively. STZ-diabetic rat show increased malondialdehyde (MDA) levels, which increased ROS production and lipid peroxidation (Ou et al., 2007). Intake of pigmented rice bran and taxifolin may ameliorate myocardial antioxidant activity and improve oxidative stress by reducing production of MDA in plasma and liver STZ-induced rats (Sun et al., 2014; Posuwan et al., 2013). Compared to the untreated group, elevated antioxidant enzyme activities were reversed by Superhongmi bran supplementation.

Plasma parameters of bone metabolism

In women, bone loss is caused by changes in estrogen secretion after menopause, and is associated with increases in the bone absorption biomarkers NTX-1 and CTX-1 and increased risk of hip fractures (Paganini-Hill et al., 1981; Chapuy et al., 1992). Osteocalcin, which is produced and excreted from bone cells, can be used as an indicator of bone absorption and regeneration (Knapen et al., 1998). Feeding Superhongmi bran extracts to menopause-induced ovariectomized rats did not significantly alter osteocalcin concentrations, except for when compared with the SHAM group (Table 4). However, greater decreases in CTX-1 and NTX-1 were observed in rats receiving Superhongmi bran, particularly in the OVX-HSH groups. These results are consistent with a previous study which showed that germinated pigmented rice may inhibit bone resorption in ovariectomized model (Chung et al., 2016). According to a study by Satué (2013), taxifolin enhances osteoblast differentiation in MC3T3-E1 cells and reduces osteoclastogenesis in RAW264.7 cells. Moreover, the female hormone 17-β-estradiol is generally low in menopausal women; 17-β-estradiol impacts bone density and cardiovascular disorders that show increased prevalence in postmenopausal women (Mendelsohn and Karas, 1999). In comparison to the OVX-AIN93M group, rats in the OVX-LSH and OVX-HSH groups showed significantly increased concentrations of 17-β-estradiol; Superhongmi may therefore increase levels of this hormone during/following menopause. However, with the exception of the sham group, there were no significant differences between the experimental groups. In conclusion, taxifolin contained in Superhongmi may protect osteoblasts cells from oxidizing stress after menopause, thus suppressing osteoclastogenesis. Moreover, through examining increased activity of anti-diabetes and antioxidant makers, we demonstrated that Superhongmi red rice may ameliorate glycemic and oxidative stress. These findings may form base data for future clinical trials.

ACKNOWLEDGEMENTS

This work was carried out with the support of Korea Institute of Planning and Evaluation for Technology in Food, Agriculture and Forestry (IPET) through High Value-added Food Technology Development Program, by Ministry of Agriculture, Food and Rural Affairs (MAFRA, No. 115018-3).

AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

REFERENCES

Aebi H. Catalase. In: Bergmeyer HU, editor. Methods in Enzymatic Analysis. 2nd ed. Academic Press, New York, NY, USA. 1974. p 673-684.

Alegre M, Ciudad CJ, Fillat C, Guinovart JJ. Determination of glucose-6-phosphatase activity using the glucose dehydrogenase-coupled reaction. Anal Biochem. 1988. 173:185-189.

Aruoma OI. Free radicals, oxidative stress, and antioxidants in human health and disease. J Amer Oil Chem Soc. 1998. 75:199-212.

Chapuy MC, Arlot ME, Duboeuf F, Brun J, Crouzet B, Arnaud S, et al. Vitamin D_3 and calcium to prevent hip fractures in elderly women. N Engl J Med. 1992. 327:1637-1642.

Chung SI, Ryu SN, Kang MY. Germinated pigmented rice (*Oryza sativa* L. cv. Superhongmi) improves glucose and bone metabo-

lisms in ovariectomized rats. Nutrients. 2016. 8:E658.
Davidson AL, Arion WJ. Factors underlying significant underestimations of glucokinase activity in crude liver extracts: physiological implications of higher cellular activity. Arch Biochem Biophys. 1987. 253:156-167.
Goufo P, Trindade H. Rice antioxidants: phenolic acids, flavonoids, anthocyanins, proanthocyanidins, tocopherols, tocotrienols, γ-oryzanol, and phytic acid. Food Sci Nutr. 2014. 2:75-104.
Ha KY, Kim YD, Lee JK, Shin HT, Noh TH, Choung JI, et al. A new aromatic and high-yielding japonica rice cultivar "Mihyangbyeo". Kor J Breed Sci. 2003. 35:251-252.
Ha KY, Lee JK, Shin HT, Lee SY, Yang BK, Kim BK, et al. A new aromatic and good grain quality japonica rice variety "Hyangnambyeo". RDA J Crop Sci. 1996. 38:54-60.
Halliwell B. Free radicals, antioxidants, and human disease: curiosity, cause, or consequence?. Lancet. 1994. 344:721-724.
Ham TH, Kwon SW, Ryu SN. A rice variety superjami 2, with large-grain and high contents of cyanidin 3-glucoside. Kor J Breed Sci. 2015. 47:299-305.
Hulcher FH, Oleson WH. Simplified spectrophotometric assay for microsomal 3-hydroxy-3-methylglutaryl CoA reductase by measurement of coenzyme A. J Lipid Res. 1973. 14:625-631.
Kim J, Lee Y, An HJ, Lee JD, Yi Y. Anti-inflammatory activities of taxifolin from *Opuntia humifusa* in lipopolysaccharide stimulated raw 264.7 murine macrophages. J Appl Biol Chem. 2015. 58:241-246.
Knapen MH, Nieuwenhuijzen Kruseman AC, Wouters RS, Vermeer C. Correlation of serum osteocalcin fractions with bone mineral density in women during the first 10 years after menopause. Calcif Tissue Int. 1998. 63:375-379.
Kronenberg F, Fugh-Berman A. Complementary and alternative medicine for menopausal symptoms: a review of randomized, controlled trials. Ann Intern Med. 2002. 13:805-813.
Kwon SW, Chu SH, Han SJ, Ryu SN. A new rice variety 'superjami' with high content of cyanidin 3-glucoside. Kor J Breed Sci. 2011. 43:196-200.
Lim SJ, Yang SJ, Song YC, Oh BG, Yeo US, Yi GH, et al. A new aromatic and glutinous japonica rice variety "Aranghyangchalbyeo". Kor J Breed Sci. 1998. 30:385.
Mackness MI, Arrol S, Durrington PN. Paraoxonase prevents accumulation of lipoperoxides in low-density lipoprotein. FEBS Lett. 1991. 286:152-154.
Matschinsky FM. Glucokinase as glucose sensor and metabolic signal generator in pancreatic β-cells and hepatocytes. Diabetes. 1990. 39:647-652.
Mendelsohn ME, Karas RH. The protective effects of estrogen on the cardiovascular system. N Engl J Med. 1999. 340:1801-1811.
Mize CE, Langdon RG. Hepatic glutathione reductase. I. Purification and general kinetic properties. J Biol Chem. 1962. 237: 1589-1595.
Moon HP, Choi YG, Jung KH, Hwang KH, Lee JH, Kim MY, et al. An aromatic semi-dwarf, high-yielding and multi-disease-resistant rice variety "Hyangmibyeo 2". RDA J Crop Sci. 1998a. 40:34-40.
Moon HP, Choi YG, Lee JH, Jung KH, Cho SY, Hwang HG, et al. A new early maturing, anthocyanin pigmented rice variety "Heugjinjubyeo". Kor J Breed Sci. 1998b. 30:383.
Moon HP, Jung KH, Choi YG, Lee JH, Hwang HG, Kim MK, et al. A new aromatic and glutinous rice cultivar "Seolhyangchalbyeo". Kor J Breed Sci. 2003. 35:241-242.
Newton KM, Reed SD, Grothaus L, Ehrlich K, Guiltinan J, Ludman E, et al. The Herbal Alternatives for Menopause (HALT) Study: background and study design. Maturitas. 2005. 52:134-146.
Osmers R, Friede M, Liske E, Schnitker J, Freudenstein J, Henneicke-von Zepelin HH. Efficacy and safety of isopropanolic black cohosh extract for climacteric symptoms. Obstet Gynecol. 2005. 105:1074-1083.
Ou SY, Jackson GM, Jiao X, Chen J, Wu JZ, Huang XS. Protection against oxidative stress in diabetic rats by wheat bran feruloyl oligosaccharides. J Agric Food Chem. 2007. 55:3191-3195.
Paganini-Hill A, Ross RK, Gerkins VR, Henderson BE, Arthur M, Mack TM. Menopausal estrogen therapy and hip fractures. Ann Intern Med. 1981. 95:28-31.
Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med. 1967. 70:158-169.
Park SZ, Kim HY, Han SJ, Ryu SN. Cyanidin 3-glucoside content in F1, F2 and F3 grains of pigmented rice Heugjinjubyeo crosses. Kor J Breed Sci. 2000. 32:285-290.
Posuwan J, Prangthip P, Leardkamolkarn V, Yamborisut U, Surasiang R, Charoensiri R, et al. Long-term supplementation of high pigmented rice bran oil (*Oryza sativa* L.) on amelioration of oxidative stress and histological changes in streptozotocin-induced diabetic rats fed a high fat diet; Riceberry bran oil. Food Chem. 2013. 138:501-508.
Qureshi AA, Sami SA, Khan FA. Effects of stabilized rice bran, its soluble and fiber fractions on blood glucose levels and serum lipid parameters in humans with diabetes mellitus types I and II. J Nutr Biochem. 2002. 13:175-187.
Rukmini C. Bioactive in rice bran and rice bran oil. In: Topham DKW, Omaye ST, Meskin MS, Bidlack WR, editors. Phytochemicals as Bioactive Agents. Technomic Publishing Co., Inc., Lancaster, PA, USA. 2000. p 213-240.
Ryu SN, Han SJ, Park SZ, Kim HR. Antioxidant activity of blackish purple rice. Kor J Crop Sci. 2006. 51:173-178.
Ryu SN. Environmental variation of taxifolin content in a rice variety 'Superhongmi'. Kor J Breed Sci. 2018. 50:45-49.
Ryu SN. Quality characteristics of new reddish brown color rice variety "Superhongmi". Kor J Crop Sci. 2015. 60:436-441.
Satué M, Arriero Mdel M, Monjo M, Ramis JM. Quercitrin and taxifolin stimulate osteoblast differentiation in MC3T3-E1 cells and inhibit osteoclastogenesis in RAW 264.7 cells. Biochem Pharmacol. 2013. 86:1476-1486.
Sun X, Chen RC, Yang ZH, Sun GB, Wang M, Ma XJ, et al. Taxifolin prevents diabetic cardiomyopathy *in vivo* and *in vitro* by inhibition of oxidative stress and cell apoptosis. Food Chem Toxicol. 2014. 63:221-232.
Wang H, Cao G, Prior RL. Oxygen radical absorbing capacity of anthocyanins. J Agric Food Chem. 1997. 45:304-309.
Yang CI, Lee KS, Choi YH, Jung KH, Jung OY, Hwang HG, et al. A new reddish brown color rice cultivar 'Hongjinju'. Kor J Breed Sci. 2011. 43:513-518.
Zhang FS, Wang QY, Pu YJ, Chen TY, Qin XM, Gao J. Identification of genes involved in flavonoid biosynthesis in *Sophora japonica* through transcriptome sequencing. Chem Biodivers. 2017. 14:e1700369.
Zhao Y, Huang W, Wang J, Chen Y, Huang W, Zhu Y. Taxifolin attenuates diabetic nephropathy in streptozotocin-induced diabetic rats. Am J Transl Res. 2018. 10:1205-1210.