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ORIGINAL ARTICLE

Characterization of bioactive agents in five types of marketed sprouts and comparison of their antihypertensive, antihyperlipidemic, and antidiabetic effects in fructose-loaded SHRs

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Abstract Hypertension, hyperlipidemia, and diabetes are important precursors of cardiovascular disease. Here, we evaluated the antihypertensive, antihyperlipidemic, and antidiabetic potential of five types of sprouts in fructose-loaded spontaneously hypertensive rats (SHRs). Powdered sprouts (PSs) were produced from mung bean, broccoli, radish, and buckwheat sprouts and germinated soybeans by lyophilization. The PSs were analyzed for nutritional composition and bioactive agents (γ-aminobutyric acid [GABA], coenzyme Q10 [CoQ10], rutin, and *myo*-inositol-1,2,3,4,5,6-hexakisphosphate [IP_6]) and functionally tested in SHRs given water containing 25 % fructose and diets containing 30 % PS for 46 days. All PSs were nutritionally rich in protein and dietary fiber. CoQ10, GABA/rutin, and GABA/IP_6 were abundant in broccoli, buckwheat, and germinated soybean PSs, respectively. Mung bean, broccoli, and buckwheat PSs caused significant reductions in heart rates and/or serum triglycerides. Mung bean PS also significantly reduced serum total cholesterol. These data supported the antihypertensive and antihyperlipidemic potential of mung bean, broccoli, and buckwheat sprouts.

Keywords Sprouts · Mung bean · Buckwheat · Broccoli · Antihyperlipidemia · Antihypertension · Fructose-loaded SHR

Highlights • Powdered sprouts (PSs) were prepared from five kinds of marketed sprouts.
• All PSs were nutritionally rich in dietary fiber and contained functional ingredients.
• Food functionalities of PSs were tested in spontaneously hypertensive rats given fructose.
• Mung bean, broccoli and buckwheat PSs significantly reduced serum triglycerides.
• Mung bean PS also significantly reduced serum total cholesterol.

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Introduction

Cardiovascular diseases are the leading causes of death and disability worldwide (Najjar et al. 2005). To prevent cardiovascular disease, it is important to eliminate the major risk factors of hypertension, hyperlipidemia, and diabetes as primary prophylaxis (Colhoun et al. 2004; Downs et al. 1998; Hansson et al. 1998). Currently, functional foods containing physiologically active dietary factors are expected to have impact of the primary prophylaxis.

Recently, the functional ingredients and functionalities of sprouts (seedling plants before true leaf unfolding) have attracted attention as potential health-promoting functional foods (Nakamura et al. 2013). Sprouts are rich in numerous vitamins and functional ingredients that accumulate during germination (Donkor et al. 2012). Plants utilize these components for rapid growth during germination and may provide human health benefits. Sprouts are also useful as functional food materials. For example, we previously reported that lactic fermented food made from buckwheat sprouts exhibited superior blood-pressure-lowering effects in spontaneously hypertensive rats (SHRs) (Nakamura et al. 2013) and that bioactive peptides could be derived from the sprout protein (Koyama et al. 2013). Sprouts, which are inexpensive and easy to obtain throughout the year, can be favorably incorporated into the daily diet and may reduce the risk of disease. Thus, studies of functional foods have examined the impact of dietary sprout consumption on disease prevention in high-calorie diets.

Nutritional ingredients in vegetables are important nutritional requirements, and some ingredients have therapeutic effects in the prevention of cardiovascular diseases (Anderson et al. 2004; Hilleboe 1957; Vaskonen 2003). Ingestion of vegetable protein in place of animal protein reduces the risk of coronary heart disease (Hilleboe 1957). Moreover, dietary fiber is thought to lower the risk of diabetes mellitus and cardiovascular disease (Anderson et al. 2004). Dietary supplementation with minerals may also reduce the risk of cardiovascular disease (Vaskonen 2003). Several potential antihypertensive, antihyperlipidemic, or antidiabetic compounds also have been identified in germinating seeds. For example, γ-aminobutyric acid (GABA) is an unusual amino acid with blood-pressure-lowering properties (Inoue et al. 2003). GABA content is increased in sprouts because it prevents acidification during germination (Bouche et al. 2003). Indeed, germinated brown rice contains 3–6 times more GABA than nongerminated brown rice (Karladee and Suriyong 2012). Moreover, coenzyme Q10 (CoQ10), a coenzyme involved in the production of ATP, possesses antihypertensive and antihyperlipidemic properties (Kumar et al. 2009). CoQ10 content is higher in cruciferous vegetables, particularly broccoli, where it is found at a concentration of approximately 0.701 mg/100 g fresh weight (FW) (Kubo et al. 2008). Therefore, cruciferous sprouts, such as radish and broccoli sprouts, are expected to have higher CoQ10 contents. Buckwheat seeds constitute a rich source of rutin in cereals. We previously reported that rutin is more abundant in buckwheat sprouts than in seeds (Koyama et al. 2011). Moreover, rutin has antihypertensive (Ajay et al. 2003) and antihyperlipidemic effects (Lee et al. 2010). Finally, *myo*-inositol-1,2,3,4,5,6-hexakisphosphate (IP_6), which exhibits antihyperlipidemic activity (Jariwalla 1999), is a common substance found in spermatophytes and functions as a source of phosphorus in seeds. Despite hydrolysis by phytase, IP_6 is expected to remain in sprouts for a short time after germination (Honke et al. 1998).

In this study, we investigated the antihypertensive, antihyperlipidemic, and antidiabetic effects of selected sprouts in SHRs in the context of a high-calorie diet. Five types of sprouts, i.e., uncolored mung bean (*Vigna radiata* cv.), green broccoli (*Brassica oleracea* cv.), radish (*Raphanus sativus* cv.), and buckwheat (*Fagopyrum esculentum* cv.) sprouts and germinated soybeans (*Glycine max* cv.), were used in this study; the first four types of sprouts (vegetable sprouts) are popular worldwide and are easy to obtain in the Japanese market, whereas germinated soybeans, which have recently become popular, are expected to have specific biological effects owing to their shorter germination period compared with that of the other sprouts. Since the nutritional and functional ingredients in sprouts are concentrated in the dry matter, we prepared these samples in the form of lyophilized powdered sprouts (PSs). First, we compared the nutritional (energy, protein, fat, carbohydrate, minerals, dietary fiber, and vitamins) and functional composition (GABA, CoQ10, rutin, and IP_6) of the five PSs. Second, we investigated the antihypertensive, antihyperlipidemic, and antidiabetic effects during fructose loading in female SHRs consuming PS-containing diets for 6 weeks. In SHRs, hypertension develops spontaneously with aging. Fructose loading to SHRs also increases body fat (Koh et al. 1985), hypertriglyceridemia (Sleder et al. 1980), and hyperinsulinemia (Sleder et al. 1980) to produce diet-induced obesity. Thus, the animals were assessed for organ weight, food and water intake, serum composition (triglycerides, cholesterol, glucose, and insulin), heart rate, systolic blood pressure (SBP), and diastolic blood pressure (DBP).

Materials and methods

Chemicals Ammonium acetate, NaCl, $MgSO_4$, KCl, $CaCl_2$, methanol, ethanol, 2-propanol, *n*-hexane, acetonitrile (HPLC grade), formic acid (HPLC grade), and D_2O were purchased from Kanto Chemical Co., Inc. (Tokyo, Japan), whereas 0.1 M HCl, CoQ10, fructose, and GABA were purchased from Wako Pure Chemical Industries (Osaka, Japan). Rutin was from Funakoshi Co., Ltd. (Tokyo, Japan), and 2-

aminoethylphosphonic acid was from Sigma-Aldrich (St. Louis, MO, USA).

Sprout cultivation Mung bean, broccoli, radish, and buckwheat sprouts and germinated soybeans were cultivated by Saladcosmo Co., Ltd. (Gifu, Japan). Soybean seeds were surface-sterilized with ozone, immersed in water (32–33 °C; 20 min), and germinated (23 °C; 20 h) in the dark to obtain germinated soybeans. Buckwheat sprouts were prepared as previously described (Nakamura et al. 2013). Broccoli and radish sprouts were similarly prepared. In brief, broccoli, radish, and buckwheat seeds were surface-sterilized with ozone and immersed in water at 32–33 °C for 5.5 h. After plating in soaking wet urethane foam for 24 h in the dark at 23 °C, the seeds were grown with culture fluid for about 10 days under shaded sunlight. Mung bean sprouts were similarly cultivated, except that cultivation was carried out under dark conditions for about 7 days after plating in soaking wet urethane.

Preparation of PSs To prepare PSs, water contained in the sprouts was eliminated by lyophilization. After heating in a water bath at 90 °C for 3 min to sterilize, the sprouts (mung bean, broccoli, radish, and buckwheat sprouts or germinated soybeans) were lyophilized for 24 h using an Eyela FDU-2000 freeze dryer (Tokyo Rikakikai CO, LTD, Tokyo, Japan) and ground using an electric mill (300 cc, 100 V, 900 w; MK; Rong Tsong Iron, Taichung, Taiwan) through≤ 400 mesh. Each prepared PS was used to analyze functional and nutritional ingredients and to prepare diets for animal experiments.

Functional ingredients of PSs GABA, CoQ10, and rutin in the PSs were measured at the Collaborated Research Center for Food Functions at the Faculty of Agriculture in Shinshu University (CREFAS). IP_6 content in the PSs was determined at the Research Center for Human and Environmental Science at Shinshu University. The measurements were performed in triplicate, and the values were expressed as means ± standard error (SE). Determination methods are briefly described below, and detailed methods are available in the Supplementary File.

GABA was measured by capillary electrophoresis as previously described (Kennedy et al. 2002). CoQ10 was analyzed by high-performance liquid chromatography (HPLC) as previously described (Mattila et al. 2000), with minor modifications. Rutin was also analyzed by HPLC as previously described (Koyama et al. 2011). IP_6 was quantified using a ^{31}P NMR method (Kemme et al. 1999).

Nutritional composition of PSs and PS diets The nutritional contents (water, protein, lipid, carbohydrate, minerals, dietary fiber, and vitamins) and total energy of each PS were analyzed by Food Research Laboratories, Japan, according to the Analytical Manual of Standard Tables of Food Composition in Japan (Science and Technology Agency, Japan 1997). The measurement methods of each component are available in the Supplementary File.

Animal diets supplemented with 30 % PS (mung bean, soybean, broccoli, and buckwheat PSs) were prepared by mixing each PS with commercial powdered feed CE-2 (CLEA Japan, Inc., Tokyo, Japan). The upper limit of the mixing ratio was 30 % PS (*w*/*w*) to prepare a homogeneous diet, and it was not possible to also add fructose to this diet. Dietary PS composition was calculated based on the nutritional composition of the CE-2 provided by CLEA Japan. The energy of PS-containing diets (kcal/100 g dry weight [DW]) was calculated from the sum of 247 kcal/100 g DW (the 70 % CE-2 energy value) and the energy value of 30 % of each PS (kcal/100 g DW) multiplied by 0.3.

Animal studies The animals were housed at Ina Research, Inc. (Nagano, Japan), where necropsies were also performed according to the guidelines for surgical and experimental procedures based on good laboratory practice (approved number: SI03282). Seven-week-old female SHRs (SHR/Izm; CLEA Japan, Inc.) were maintained in individual cages (one rat per cage) in a temperature-controlled room (23±2 °C) with a 12-h light/dark cycle (lights from 7 AM to 7 PM) and free access to tap water and food (CE-2 powder; CLEA Japan Inc.). The animals were acclimatized for 10 days until the beginning of the experiment. One day before the experiment, the animals (aged 8 weeks) were randomly divided into six groups of six animals each: a normal group given CE-2 and tap water; a control group given CE-2 and a 25 % (*w*/*w*) fructose solution (368 kcal/100 g) for energy loading; and four test groups given 30 % (*w*/*w*) PS mixed with CE-2 and the fructose solution. The mixed rate was determined based on results of nutritional composition analysis of PSs. During the 46-day experiment, body weights and food and water consumption were determined every 3 or 4 days. Energy intake in the normal group (kcal/day) was calculated by multiplying food consumption (g/day) by 3.53 (the energy density in kcal/g of the normal diet). Energy intake in other groups (kcal/day) was calculated from the sum of the food energy intake value, determined by multiplying food consumption (g/day) by the energy density (kcal/g for each diet), and water energy intake value, determined by multiplying fructose solution consumption (g/day) by 0.92 (the energy density in kcal/g of the fructose solution).

Blood pressures and heart rate: One day before and 22 and 42 days after the beginning of PS feeding, SBP and DBP were measured by the tail cuff method with a Softron BP98A (Softron Co. Ltd., Tokyo, Japan). Before measurement, the

Table 1 Functional ingredients in PSs (mg/100 g DW)

	GABA	CoQ10	Rutin	IP_6
Mung bean PS	61.6±1.1[a]	2.60±0.08[a]	N.D.	N.D.
Broccoli PS	30.7±0.8[b]	15.3±0.8[b]	76.9±0.8[b]	N.D.
Radish PS	18.7±0.3[c]	9.24±0.32[c]	33.0±0.3[c]	N.D.
Buckwheat PS	144.7±2.0[d]	2.97±0.02[a]	768.6±2.6[d]	N.D.
Germinated soybean PS	116.4±1.7[e]	1.91±0.22[a]	78.7±1.2[b]	459.3±4.5

DW dry weight, *N.D.* not detected, *CoQ10* coenzymeQ10, *GABA* γ-aminobutyric acid, *IP_6* *myo*-inositol-1,2,3,4,5,6-hexakisphosphate

Different letters indicate significant differences between PS types ($p<0.05$, one-way ANOVA followed by Tukey's test)

rats were kept at 38 °C for 10 min to facilitate detection of the pulsations of the tail artery. Results at each time point were expressed as the mean±SE.

Serum triglycerides, cholesterol, glucose, and insulin: At 1, 23, and 43 days after the beginning of experiment, 1.0 mL blood was drawn from the carotid artery of each rat under ether anesthesia. The samples were allowed to coagulate at room temperature for 0.5 h and at 4 °C for 16 h, and samples were then centrifuged at 1,200×*g* for 20 min at 4 °C. The separated serum was frozen and stored at −80 °C until assayed for triglyceride and total cholesterol as lipid indicators and glucose and insulin as antidiabetic indicators. The triglycerides, total cholesterol, and glucose in the serum were measured by enzymatic assays on a Hitachi 7150 automatic analyzer (Hitachi Ltd., Tokyo, Japan). On day 43, serum insulin was measured using an Insulin-Rat T ELISA kit (Shibayagi Co. Ltd., Gunma, Japan) on a MTP-32 microplate reader (Corona Electric Co., Ltd., Ibaraki, Japan). On day 46, the rats were euthanized by exsanguination via the abdominal vena cava under ether anesthesia, and the white adipose tissues (WATs) around the uterus, heart, liver, and right kidney were excised and weighed after autopsy.

Statistical analysis Contents of functional ingredients in PSs were compared by one-way analysis of variance (ANOVA) followed by Tukey's test to determine the level of significance ($p<0.05$). In animal tests, comparisons among the normal, control, and test groups were verified for homoscedasticity using *F*-tests followed by Student's *t*-tests. Student's *t*-tests were also performed to compare the heart rate, SBP, and DBP before the experiment and at days 22 or 42 during the experiment. The significance levels were $p<0.05$ for the *F*-test and $p<0.05$ and $p<0.01$ for the *t*-tests.

Results

We prepared the five types of PSs (mung bean, broccoli, radish, buckwheat, and germinated soybean PSs) and characterized them by nutritional and functional component analyses and animal studies.

Nutritional and functional properties

During the germination process, substances stored in the seed are decomposed and transformed into plant components. These chemical alternations progress quickly and dramatically in sprouts. Therefore, sprouts are expected to have superior nutritional value than seeds due to the formation of these functional ingredients. Thus, we analyzed the functional and nutritional ingredients of each PS.

Functional ingredients in PSs The five PS preparations exhibited distinct compositions of bioactive ingredients (Table 1). First, all PSs contained different concentrations of GABA, in the following order (highest to lowest): buckwheat

Table 2 Nutritional compositions (vitamins: mg/100 g DW, others: g/100 g DW) and energies (kcal/100 g DW) of the PSs

	Energy	Water	Protein	Lipid	Carbohydrate	Minerals	Dietary fiber	VB_1	VB_2	VC
Mung bean PS	330	2.3	31.0	3.5	26.6	2.7	33.9	0.7	0.4	101
Broccoli PS	356	3.8	33.9	13.4	9.9	8.7	30.3	1.2	1.3	226
Radish PS	363	3.6	36.0	12.0	13.5	6.2	28.7	1.2	1.5	315
Buckwheat PS	340	3.2	34.9	6.3	21.5	5.4	28.7	1.2	1.2	90
Germinated soybean PS	461	3.1	38.7	25.0	11.6	4.4	17.2	0.9	0.3	3

DW dry weight, *VB_1* vitamin B_1, *VB_2* vitamin B_2, *VC* vitamin C

Table 3 Nutritional compositions (g/100 g DW) and energies (kcal/100 g DW) of control and PS-containing diets

	Energy	Water	Protein	Lipid	Carbohydrate	Minerals	Dietary fiber
CE-2 (control)	353	8.5	24.9	4.8	50.4	7.0	4.4
Mung bean PS	346	6.6	26.7	4.4	43.3	5.7	13.3
Broccoli PS	354	7.1	27.6	7.4	38.3	7.5	12.2
Buckwheat PS	349	6.9	27.9	5.3	41.7	6.5	11.7
Germinated soybean PS	385	6.9	29.0	10.9	38.8	6.2	8.2

DW dry weight

> soybean > mung bean > broccoli > radish (ANOVA; F=1138.5; $p=3.0\times10^{-13}$). In contrast, CoQ10 was 3–8 fold more abundant in broccoli and radish PSs than in the other PSs (ANOVA; F=143.4; $p=8.7\times10^{-9}$). Buckwheat PSs was at least 10–20 fold more enriched in rutin than in broccoli, radish, and soybean PSs (ANOVA; F=36842.3; $p=6.8\times10^{-17}$), but was not detected in mung bean PSs. Finally, the only source of IP_6 was soybean PSs. Therefore, from these data, we expected that the animals would respond differently to each of the five PS preparations.

Nutritional composition of PSs We assessed each PS to determine energy, protein, lipid, minerals, carbohydrate, dietary fiber, and vitamins B_1, B_2, and C (Table 2). The two major nutritional ingredients in PSs of the vegetable sprouts were protein and dietary fiber, with averages of 34.0 and 30.4 %, respectively. The average energy of PSs was 370.0 kcal/100 g DW. The energy value in each PS was dependent on the lipid content, which varied widely as follows (highest to lowest): soybean > radish > broccoli > buckwheat > mung bean. Minerals were a minor nutrient. The remaining carbohydrate factor indicated assimilative sugars and/or unused carbohydrates in PSs. The range of contents also varied widely as follows (highest to lowest): mung bean > buckwheat > radish > soybean > broccoli.

Table 2 also shows the selected vitamin contents. Vegetable PSs were particularly abundant in vitamin C with various concentrations as follows (highest to lowest): radish > broccoli > mung bean > buckwheat > soybean. All PSs contained similar levels of vitamins B_1 and B_2. Collectively, the PSs were rich in protein and dietary fiber. The PSs prepared from the vegetable sprouts were rich in vitamin C in addition to the two major nutrients.

Functionality of each PS in animals

The effects of mung bean, germinated soybean, broccoli, and buckwheat PS consumption were investigated in rats. We expected that GABA and dietary fiber would produce antihypertensive and antihyperlipidemic effects, respectively. CoQ10, rutin, and/or IP_6 were also expected to have antihypertensive, antihyperlipidemic, and/or antidiabetic effects. Because the mung bean and germinated soybean PSs were poor in minerals, mixing 30 % of each PS in the normal diet was sufficient for rodents. The ratios of all PSs were unified for equivalent determinations of food functionality. Thus, we examined the effects of a diet containing each PS at a 30 % ratio, which was the upper limit required to maintain the homogeneity of the diet, on hypertension, hyperlipidemia, and diabetic symptoms in fructose-loaded SHRs. The nutritional composition and

Table 4 Individual average food and fructose solution consumption, energy intake, and changes in body weight

Test group	Food consumption (g/day)	Fructose solution consumption[a] (g/day)	Energy intake (kcal/day)	Body weight (g)	
				Day 1	Day 46
Normal	13.8±0.1**	22.3±0.6**	48.8±0.5*	141±2	174±2*
Control (CE-2)	7.9±0.1	26.9±0.9	52.0±1.1	140±3	183±2
Mung bean PS	7.4±0.2	27.7±0.6	51.0±0.8	141±2	185±4
Broccoli PS	7.3±0.2	24.9±0.7	48.9±0.6	143±2	181±2
Buckwheat PS	8.1±0.3	27.1±0.6	53.2±1.3	142±1	184±3
Germinated soybean PS	7.5±0.1	24.0±0.8	50.4±0.6	142±2	184±1

[a] Tap water was provided to the normal group, and 25 % (*w/w*) fructose solution was provided to the other groups

Significant differences compared with the control: * $p<0.05$, ** $p<0.01$ (Student's *t*-test)

Table 5 Weights of body organs and adipose tissues

Tissue weight	Normal		Control		Mung bean PS		Broccoli PS		Buckwheat PS		Germinated soybean PS	
	Absolute (g)	Relative† (%)	Absolute (g)	Relative† (%)	Absolute (g)	Relative[a] (%)	Absolute (g)	Relative[a] (%)	Absolute (g)	Relative[a] (%)	Absolute (g)	Relative[a] (%)
Heart	0.81±0.01	0.47±0.01	0.84±0.02	0.46±0.004	0.85±0.02	0.46±0.004	0.86±0.01	0.48±0.004	0.84±0.02	0.45±0.004	0.84±0.01	0.46±0.004
Kidney	0.70±0.01	0.40±0.004	0.69±0.01	0.38±0.004	0.71±0.01	0.38±0.01	0.71±0.01	0.39±0.01	0.68±0.01	0.37±0.01	0.70±0.01	0.38±0.01
Liver	7.25±0.13	4.16±0.04	8.33±0.22§§	4.57±0.09§§	8.22±0.20	4.44±0.07	9.58±0.16**	5.29±0.09**	8.01±0.20	4.35±0.04	7.99±0.18	4.35±0.08
WAT	1.43±0.13	0.82±0.07	2.65±0.25§§	1.46±0.15§§	2.64±0.21	1.42±0.09	1.88±0.20*	1.04±0.11*	2.63±0.18	1.43±0.09	2.55±0.23	1.39±0.13

Tap water was provided to the normal group, and 25 % (*w*/*w*) fructose solution was provided to the other groups

WAT white adipose tissue

In each group, the left column is the absolute weight, and the right column is the relative weight

[a] Percent of body weight

Significance: * p<0.05, ** p<0.01 (Student's *t*-test, versus the control group)

Significance: §§ p<0.01 (Student's *t*-test, versus the normal group)

Table 6 Averages and changes in blood pressures and heart rates

	Day	Normal		Control		Mung bean PS		Broccoli PS		Buckwheat PS		Germinated soybean PS	
SBP (mmHg)	−1	176.8±5.9	–	178.0±5.5	–	168.2±10.2	–	172.5±6.5	–	174.7±4.6	–	178.2±5.4	–
	22	178.8±7.5	2.0±6.9	200.0±6.5	22.0±6.7§§	196.0±5.5	27.8±9.5§	185.8±5.6	13.3±10.7	190.0±7.5	15.3±6.1§	185.2±4.0	7.0±5.2
	42	181.7±8.1	4.8±9.2	196.8±3.1	18.8±4.4§§	208.0±2.3	39.8±8.3§§	192.3±5.8	19.8±7.7§	201.3±4.5	26.7±7.8§§	199.0±10.6	20.8±13.4
DBP (mmHg)	−1	126.5±5.2	–	137.0±3.0	–	134.3±7.4	–	128.5±2.3*	–	133.7±6.5	–	139.8±5.3	–
	22	141.7±6.4	15.2±5.3§	150.0±3.0	13.0±3.2§§	150.7±3.4	16.3±8.5	145.7±4.8	17.2±5.5§	142.8±7.6	9.2±10.0	141.8±6.0	2.0±7.4
	42	135.0±8.0	8.5±8.9	154.8±8.0	17.8±7.7§	161.3±5.0	27.0±8.2§§	156.8±6.0	28.3±7.4§§	161.3±5.2	27.7±9.4§	155.8±6.2	16.0±8.4
HR (bpm)	−1	445.8±9.6	–	452.5±5.8	–	468.0±5.1	–	434.5±10.4	–	448.2±12.1	–	440.5±6.5	–
	22	469.5±9.0	23.7±11.0	494.0±7.8	41.5±10.5§§	453.3±9.1**	−14.7±9.5	442.3±12.5**	7.8±19.7	473.5±6.6	25.3±10.1§	488.0±9.4	47.5±11.8§§
	42	460.2±7.6*	14.3±7.4	500.5±11.8	48.0±11.8§§	474.2±12.4	6.2±13.0	443.3±8.2**	8.8±11.4	503.3±12.9	55.2±6.8§§	500.3±4.7	59.8±7.4§§

Tap water was provided to the normal group, and 25 % (*w*/*w*) fructose solution was provided to the other groups

SBP systolic blood pressure, *DBP* diastolic blood pressure, *HR* heart rate

In each group, the left column is the average value per day, and the right column indicates changes from day -1

Significance: $^{*}p$<0.05, $^{**}p$<0.01 (Student's *t*-test, versus the control group)

Significance: § p<0.05, §§ p<0.01 (Student's *t*-test, versus values at day -1)

Table 7 Triglyceride (TG), total cholesterol (TC), glucose (Glc), and insulin (Ins) in serum samples

Parameter	Day	Normal	Control	Mung bean PS	Broccoli PS	Buckwheat PS	Germinated soybean PS
TG (mg/dL)	1	72.2±10.9	63.8±3.1	70.6±8.2	92.1±4.4**	75.0±5.3	65.0±6.7
	23	56.5±2.2**	144.8±14.5	102.4±9.0*	96.3±6.8*	103.9±6.9*	111.2±14.3
	43	69.8±4.7**	113.1±10.9	114.6±10.9	105.2±12.3	121.6±9.3	94.8±12.7
TC (mg/dL)	1	72.3±1.3	75.0±1.6	72.5±2.3	74.5±2.2	77.0±2.9	77.5±1.4
	23	73.0±1.6**	92.4±1.1	82.7±1.6**	100.3±1.9	93.7±1.4	89.8±1.2
	43	77.0±1.7**	100.3±3.7	84.5±1.8**	96.3±3.1	98.2±3.4	97.3±1.2
Glc (mg/dL)	1	123.5±5.3	130.5±2.2	127.3±3.1	131.2±3.1	123.3±3.1	124.5±3.6
	23	117.8±2.9*	132.3±4.9	144.2±8.7	134.5±2.9	132.8±7.9	129.7±7.0
	43	103.8±2.6	126.7±10.2	115.3±4.4	130.5±3.7	130.0±6.9	112.8±3.3
Ins (pg/mL)	43	705.0±104.9**	1236.8±81.8	1272.8±59.2	1097.3±68.4	1205.8±125.5	1079.0±131.1

Tap water was provided to the normal group, and 25 % (*w*/*w*) fructose solution was provided to the other groups

Significance: * $p<0.05$, ** $p<0.01$ (Student's *t*-test)

energy of control and PS-containing diets are shown in Table 3. The test rats in each group were fed the PS diet ($n=6$ each) and the normal and control rats were fed the normal commercial diet ($n=6$ each) for 46 days. Radish PSs were not used because their nutritional composition, including dietary fiber, was similar to that of broccoli PSs, whereas the concentrations of functional components, i.e., GABA and CoQ10, were lower.

We also assessed the heart rate, SBP, DBP, triglycerides, cholesterol, glucose, and insulin, as well as organ and WAT weights. In female SHRs, we can specifically investigate fat accumulation by weighing the WAT around the uterus, which is an indicator of obesity (Hirako et al. 2012). No behavioral abnormalities were observed during the experimental period, and there were no visible abnormalities upon dissection at the end of the experiment.

Food and water consumption, and body and adipose tissue weight Table 4 shows the average food and fructose solution consumption, energy intake, and changes in body weight. In the normal groups, because of only tap water was given, daily energy intake ($p<0.05$) was significantly lower than that of control group, despite significantly higher ($p<0.01$) food consumption. Thus, the 25 % fructose solution produced high-calorie loading in the control and test groups. sThe body weights of individual SHRs in the normal group were the same as those in other groups on day 1, despite their significantly reduced body weights on day 46 ($p<0.05$).

In the control and test groups, diet composition had no effect on daily food or fructose solution consumption or on daily energy intake (Table 4), despite the variety of energy values and nutritional contents of the experimental diets. Dietary composition had no effect on total weight gain over the 46-day experiment. The average daily food consumption of individual rats was within the range of 7.3–8.1 g/day in each diet, with a relative standard deviation (RSD) of 4.7 %. The average individual daily consumption of 25 % fructose solution was within 24.0–27.7 g/day, with a RSD of 6.1 %. Daily individual energy intake was calculated from the average diet and fructose consumption; energy intake averaged 48.9–53.2 kcal/day with a RSD of 3.6 %. The average body weight in the control group increased from 140±3 g on day 1 to 183±2 g on day 46. Average body weight also increased in the test groups and did not differ significantly from those in the control group. The average individual body weights of control and test SHRs on day 46 ranged from 181 to 185 g, with a RSD of 0.83 %. These data showed that each diet, including the commercial diet that was used as a control, provided the same energy level and that all animals in control and test groups gained similar amounts of body weight during the experiment.

Table 5 shows the absolute and relative weights for the heart, right kidney, liver, and WAT on day 46. Both the absolute and relative weights (ratio of organ to body weight) of the liver and WAT in the control group were significantly higher ($p<0.01$) than those in the normal group, indicating that the fructose solution caused symptoms of obesity with high calorie consumption. Organ and tissue weights did not differ between the control and test animals, with the exception of the broccoli PS group, in which liver weight was significantly higher ($p<0.01$) by 15.0 % (absolute weight) and 0.72 % (relative weight), and the WATs weight around the uterus, which was significantly lower ($p<0.05$) by 29.1 % (absolute weight) and 0.42 % (relative weight).

Blood pressures and heart rate On days -1, 22, and 42, SBP, DBP, and heart rate were measured noninvasively by the tail-cuff method (Table 6). Although blood pressures increase with aging in SHRs, values in female rats are expected to be maintained from 8 to 14 weeks of age, the period of the experiment (Tsuchikura 2010). Thus, changes in these parameters in the normal group were not significant during the experimental period, with the exception of DBP on day 22 (15.2 mmHg increase from -1 day, $p<0.05$). In contrast, blood pressures and heart rate were significantly elevated in the control group on days 22 and 42 versus that at the beginning of the experiment ($p<0.01$ or 0.05). Fructose consumption caused a significant increase in blood pressure and heart rate in female rats, in addition to the aging-related increase in blood pressure. In the mung bean and broccoli PS groups, however, heart rate did not increase significantly by days 22 and 42 compared with that on day 1, and was significantly lower on day 22 in rats consuming the mung bean PS diet ($p<0.01$) and on days 22 and 42 in rats consuming the broccoli PS diet ($p<0.01$) in comparison to the controls. In the germinated soybean PS group, SBP and DBP did not significantly increase on days 22 and 42 compared with those on day -1 and showed lower, although not significant, values than the control group on day 22 (SBP: −14.8; DBP: −8.2 mmHg). On day 22, the SBP of rats in the broccoli PS group and the DBP of rats in the buckwheat PS group also did not significantly increase compared with those on day -1, with lower, although not significant, values than the control group (SBP of broccoli PS group: -14.2; DBP of buckwheat PS group: −7.2 mmHg). These data suggested that mung bean and broccoli PSs suppressed the elevation in heat rate and that germinated soybean, broccoli, and buckwheat PSs may suppress the elevation in blood pressure in fructose-loaded SHRs.

Serum triglycerides, cholesterol, glucose, and insulin Triglycerides, total cholesterol, and glucose were assayed in serum samples on days 1, 23, and 43, and insulin was measured on day 43 (Table 7). First, biochemical test results in the control groups were compared to those in the normal group to confirm symptoms of hyperlipidemia and hyperinsulinemia caused by fructose loading. Serum triglyceride and cholesterol levels on days 23 and 43, glucose levels on day 23, and insulin levels on day 43 were significantly higher ($p<0.01$ or 0.05) in the controls than in the normal group (Table 7). Thus, fructose loading produced symptoms of hyperlipidemia and hyperinsulinemia in the control group, and we were then able to evaluate the effects of PSs on these symptoms by measuring changes in these parameters in the test groups.

Serum triglycerides rapidly increased in the control group by 2.3 fold (from 63.8 to 144.8 mg/dL) until day 23 and then decreased to 1.3 fold (to 113.1 mg/dL) by day 43. On day 23, the values in the mung bean PS, buckwheat PS, and broccoli PS groups were significantly lower ($p<0.05$) than those in the control group, although this difference was not significant on day 43. Serum triglycerides did not decrease in the germinated soybean PS group. Serum total cholesterol gradually increased in the control group to 1.3 fold by the end of the experiment. In the mung bean PS group, total cholesterol content was significantly lower ($p<0.01$) than in the control group on days 23 and 43. Serum glucose was maintained in the control group and was not different from those in the PS groups. The insulin level at day 43 did not differ between the control and PS groups. These results demonstrated the serum triglyceride- and total cholesterol-lowering effects of dry mung bean sprouts and the serum triglyceride-lowering effects of dry broccoli and buckwheat sprouts in fructose-loaded SHRs.

Discussion

In this study, we prepared five types of PSs, which were lyophilized products of the sprouts. Nutritional analysis showed that the PSs were generally rich in protein and dietary fiber. Vegetable protein and dietary fiber have been shown to be associated with a lower risk of diabetes mellitus and cardiovascular disease (Anderson et al. 2004; Hilleboe 1957). Moreover, GABA, CoQ10, and rutin have antihypertensive effects (Inoue et al. 2003; Kumar et al. 2009; Lee et al. 2010), and CoQ10, rutin, and IP_6 have antihyperlipidemic effects (Jariwalla 1999; Kumar et al. 2009; Lee et al. 2010). We detected GABA in all PS preparations, with the highest levels found in buckwheat and germinated soybean PSs. These data are consistent with earlier reports of GABA content increasing after germination in buckwheat and soybeans (Lin et al. 2008). CoQ10 was detected in all PSs and was highest in broccoli PSs. This bioactive agent was only detected starting on day 2 after germination (data not shown). Rutin was particularly concentrated in buckwheat PSs and was not detected in mung bean PSs. We recently reported that the rutin content of buckwheat sprouts increased during germination (Koyama et al. 2011). IP_6 was only detected in germinated soybean PSs. Importantly, germinated soybean PSs still contain IP_6 because of the short germination period of this product (Honke et al. 1998), and IP_6 was not detected in the vegetable sprouts with longer germination periods. Therefore, this study was uniquely designed to compare PSs predicted to produce distinct effects in SHRs.

To test the antihypertensive, antihyperlipidemic, and antidiabetic effects of the PSs, we performed an animal experiment with female SHRs for 46 days under fructose energy load. Mung bean and broccoli PSs caused significant decreases in heart rate, and germinated soybean, broccoli, and buckwheat PSs tended to suppress the elevation in blood pressure observed in the control group. However, these antihypertensive effects did not appear to be the main functionalities of the PSs because of the inconclusive statistical results.

Antihyperlipidemic effects were observed in rats fed mung bean PS, broccoli PS, and buckwheat PS diets. The common functional component in these PSs was dietary fiber, which has antihyperlipidemic effects, including reduction of blood triglycerides and cholesterol, as previously described (Brownlee 2011).

Rutin-enriched buckwheat PSs significantly decreased triglycerides on day 23. A previous study reported that intake of buckwheat leaf and flower parts improved lipid profiles in rats fed a high-fat diet by lowering plasma triglyceride concentrations (Lee et al. 2010). This report suggested that these effects could be attributed to the abundance of rutin in buckwheat plants. Rutin has been reported to reduce serum triglyceride significantly by suppressing oxidative stress induced by a high-fat diet (Hsu et al. 2009). Therefore, we hypothesized that rutin was responsible for the reduced triglyceride levels observed in the energy loaded SHRs consuming the buckwheat PS diet.

The broccoli PS diet reduced the heart rate by 40–50 bpm at day 22. This diet also caused a significant decrease in triglycerides after 23 days. At the end of the experiment, a significant decrease in WAT weight and a significant increase in liver weight were found in the broccoli PS group. No hepatic fat accumulation was found, suggesting that the increased liver weight was mainly due to increased liver cell proliferation. Thus, peroxisome proliferator-activated receptor α (PPARα) may contribute to the effects of broccoli PS. PPARα is a nuclear receptor that regulates fatty acid metabolism, primarily in the liver, heart, and kidney (Aoyama et al. 1998). In rodents, PPARα activation enhances fatty acid β-oxidation and lipoprotein catabolism, thus reducing serum triglycerides. PPARα activation also enhances fatty acid uptake into hepatocytes and other cells to facilitate triglyceride hydrolysis. The subsequent fatty acid release from WAT results in a decrease in the size of the WAT. Furthermore, PPARα activation accelerates cell cycle progression and cell proliferation in the rodent liver, leading to hepatomegaly (Tanaka et al. 2008). Interestingly, the expression of PPARα in liver cells is stimulated by CoQ10 (Schmelzer et al. 2010). Therefore, the abovementioned effects of broccoli PS may be explained by CoQ10-mediated PPARα activation.

Finally, we found that mung bean PSs caused a significant reduction in serum cholesterol and triglyceride levels. Dietary fibers extracted from immature plants including mung beans produce a plasma cholesterol-lowering effect in rats (Nishimura et al. 2000). This report showed that total dietary fiber negatively correlates with plasma cholesterol, whereas protein content does not. The most abundant functional component in mung bean PSs was dietary fiber, and the abundance of other functional ingredients was poor. We suggest that the dietary fiber in mung bean PS was responsible for the serum triglyceride- and cholesterol-lowering effects observed here. D-Glucaric acid is another candidate cholesterol-lowering compound in mung bean PS and has been shown to have similar effects in Sprague–Dawley rats fed a high-fat purified diet (Walaszek et al. 1996). Thus, we suggest that the high dietary fiber content and presence of cholesterol-lowering compounds, such as D-glucaric acid, may have caused the reduction in cholesterol in the mung bean PS-fed group. However, we have not yet identified the bioactive agent(s) responsible for the antihyperlipidemic effects of mung bean PSs.

Conclusion

In this report, we demonstrated the unique in vivo antihyperlipidemic effects of mung bean, broccoli, and buckwheat PSs in female SHRs maintained on a high-calorie diet. In order to use the PSs as functional food materials, more research is needed to elucidate the antihyperlipidemic mechanisms and active compound(s). We are currently investigating the effects of broccoli, mung bean, and buckwheat PSs on the expression of genes related to lipid metabolism, including PPARα.

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