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Original article

Glycative stress-alleviating effects of black beans

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Abstract

***Objective*:** Glycation is a non-enzymatic reaction in which reducing sugars and derived aldehydes bind to amino groups of proteins, leading to the formation of advanced glycation end products (AGEs). AGEs accumulate in tissues with aging, causing inflammation, discoloration, and functional deterioration, and are associated with the onset and progression of lifestyle-related diseases. This study investigated the glycative stress-alleviating effects of black soybeans.

***Methods*:** Seven commercially available black soybean varieties, one yellow soybean variety, 12 isoflavones, and cyanidin-3-glucoside (C3G) were used. Beans were hot-water-extracted at 80°C for 1 hour as whole-bean samples or separated into seed coats and cotyledons prior to extraction. Glycation inhibitory effects were evaluated using a human serum albumin (HSA)–glucose glycation model by measuring suppression of fluorescent AGE formation. Antioxidant activity was assessed by the DPPH method. Isoflavone and C3G contents were quantified by reversed-phase HPLC. In addition, α-glucosidase inhibitory activity and the effect of black bean intake on postprandial blood glucose levels were examined.

***Results*:** All soybean extracts inhibited fluorescent AGE formation, with black soybeans showing stronger effects than yellow soybeans. Among black soybeans, Kurosengoku exhibited the highest inhibition rate (85.3 ± 2.5 %). Seed coat extracts showed significantly stronger anti-glycation activity than cotyledon extracts, with inhibition rates of up to 99.7 ± 0.28 %. All black soybean varieties demonstrated antioxidant activity, with a maximum of 286.1 μmol Trolox equivalents/L (7.5 mg/mL solids). Isoflavones were more abundant in seed coats than cotyledons, while C3G was detected only in seed coats.

***Conclusion*:** Black soybeans exhibit both anti-glycation and antioxidant properties, suggesting their potential as a dietary strategy to alleviate glycative stress.

***KEY WORDS*:** black beans, glycation, advanced glycation end products (AGEs), antioxidant activity, cyanidin-3-glucoside

Introduction

Reducing sugars such as glucose and fructose are essential nutrients for life. However, these reducing sugars can non-enzymatically bind to amino groups on proteins in vivo. This reaction, called glycation, leads to the production of glycation intermediates and advanced glycation end products (AGEs)[1]. During this process in the body, postprandial hyperglycemia (especially blood glucose spikes of ≥ 140 mg/dL) disrupts the equilibrium between open-chain and cyclic forms of glucose. As a result, exposed aldehyde groups (-CHO) undergo chain reactions with surrounding molecules, generating various sugar-derived short-chain aldehydes. These then react with free fatty acids to generate additional lipid-derived aldehydes[2, 3]. We have termed this phenomenon the "aldehyde spark"[4].

In foods, the Maillard reaction begins with the reaction of the exposed aldehyde group of ring-opened glucose with amino groups on proteins or peptides, forming Schiff bases and Amadori compounds. Carbohydrate-derived aldehydes (GO, MGO, 3DG) generated during this process act as intermediates that produce AGEs via multiple pathways. In humans, however, the large amounts of aldehydes produced by the aldehyde spark not only trigger carbonylation reactions but also act as intermediates that convert endogenous proteins to AGEs more rapidly than the classic Maillard reaction. Blood aldehydes react with circulating components and vascular endothelial surfaces, and also pass through cell membranes to modify intracellular and tissue proteins, converting them into AGEs and impairing their physiological functions.

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With aging, AGEs accumulate in various tissues and organs, causing inflammation, discoloration, and a decline in physiological function. They are implicated in the onset and progression of lifestyle-related diseases such as diabetic complications, eye diseases [5], skin aging [6], osteoporosis [7], Alzheimer's disease [4], and arteriosclerosis [9]. The concept that aging and disease risk are promoted by glycation reactions is referred to as glycative stress. In recent years, glycative stress has been recognized as one of the risk factors that accelerate aging.

Black beans ('Kuromame') are a type of soybean (*Glycine max* (L.) Merrill) that contain isoflavones, a class of phytoestrogens, and previous research has shown that they can suppress oxidative stress [10]. Approaches to suppress glycative stress include reducing hyperglycemia, inhibiting glycation reactions, and promoting the degradation and excretion of glycation products [11,12]. In this study, we focused on the glycative stress-alleviating effects of black beans and examined their ability to inhibit the formation of fluorescent AGEs, their DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity, their α-glucosidase inhibitory effect, and the effect of black bean intake on suppression of postprandial increases in blood glucose levels.

Methods

(1) Reagents

Human serum albumin (HSA, lyophilized powder, ≥ 96%, agarose gel electrophoresis) was purchased from Sigma-Aldrich Co. LLC (St. Louis, MO, USA) and used as a model protein for the glycation reaction. All other reagents were special grade or HPLC grade and were purchased from Fujifilm Wako Pure Chemical Corporation (Osaka, Japan) or Nacalai Tesque, Inc. (Kyoto, Japan).

(2) Sample preparation

Seven commercially available black soybean varieties, one yellow soybean variety (***Table 1***), 12 isoflavones, and cyanidin-3-glucoside (C3G) were used as samples. The bean samples were ground in a food processor and extracted with hot water at 80 °C for 1 hour to obtain extracts. In a separate procedure, beans were divided into seed coats and cotyledons, and extracts of each part were obtained using the same method. The solids concentration (mg/mL) of each extract was calculated by placing 5 mL of the sample extract on an aluminum tray and weighing it before and after drying at 120 °C for 120 minutes. For the experiments, whole-bean extracts were diluted with purified water to solids concentrations of 3mg/mL, 7.5 mg/mL, and 10 mg/mL, while extracts from individual bean parts were adjusted to 3 mg/mL. Three glycosides and three aglycones were completely dissolved in dimethyl sulfoxide and ethanol, respectively, and then diluted with 0.01 mol/L phosphate buffer (pH 2.6). The three acetylated glycosides and three malonylated glycosides were dissolved in ethanol before use. Aminoguanidine (AG) was prepared at 1 mg/mL in purified water. Acarbose was prepared at 3.3 mg/mL in purified water.

(3) Verification of glycation inhibitory effect

To verify the inhibitory effect on glycation, an HSA-glucose glycation reaction model was used [13]. The glycation reaction solution was prepared using 0.1 mol/L phosphate buffer (pH 7.4), 2.0 mol/L glucose, and 40 mg/mL HSA. The final concentrations in the reaction mixture were 0.05 mol/L phosphate buffer, 0.2mol/L glucose, and 8 mg/mL HSA. The sample solution was added to the reaction mixture at one-fifth of the final volume. The reaction mixture was then incubated at 60 °C for 40 hours, after which AGE-derived fluorescence (excitation wavelength 370 nm/emission wavelength 440 nm) was measured using a microplate reader. A control (reference, ref) was prepared by adding an equal volume of purified water instead of the black bean sample and incubating under the same conditions. A positive control for inhibition of fluorescent AGE formation was prepared by adding AG (1 mg/mL) at the same volume instead of sample. The fluorescence intensity was expressed as a relative value, with 5 μg/mL quinine sulfate set to 1,000 arbitrary units.

The fluorescent AGE formation inhibition rate was calculated using the following formula:

Fluorescent AGE formation inhibition rate (%)
= 100 – {[(sample Glucose(+) – sample Glucose(–)) / (ref Glucose(+) – ref Glucose(–))] × 100}

Table 1. Sample profile.

No.	Sample name	Production area	Manufacture/Seller	
1	Tanbabudoukuromame	Kyoto	Kyo no Kuromame Kitao	Black beans
2	Hikarikurodaizu	Hokkiado	JAJA Obihiro Kawanishi Agricultural Cooperative	Black beans
3	Hakodatekuromame	Hokkaido	TOMIZAWA SHOTEN CO., LTD.	Black beans
4	Tanbakuromame (bittyu) kuronowarai	Okayama	TOMIZAWA SHOTEN CO., LTD	Black beans
5	Gannkuimame	Iwate	TOMIZAWA SHOTEN CO., LTD	Black beans
6	Tanbakuromame (tobikiri)	Hyogo	TOMIZAWA SHOTEN CO., LTD	Black beans
7	Kurosengoku	Hokkaido	TOMIZAWA SHOTEN CO., LTD	Black beans
8	Daizu	Hokkaido	Hokuren Federation of Agricultural Cooperatives	Soybeans

In addition, IC_{50} values were calculated by generating a calibration curve from the fluorescent AGE formation inhibition rates at multiple sample concentrations, obtaining a regression line, and determining the x-value when y = 50. The concentration that inhibited 50 % of fluorescent AGE formation (mg/mL) was defined as the IC_{50}.

(4) Verification of DPPH radical scavenging activity

DPPH radical scavenging activity was measured using Trolox as the standard, and the results were expressed as Trolox equivalents (Eq)[14,15]. Trolox (0–16 nmol/assay) and sample (25–100 µL/assay) were dispensed into a 96-well plate, followed by the addition of 200 mmol/L MES buffer and 800 µmol/L DPPH solution. After incubation at room temperature for 20 minutes, absorbance at 520 nm was measured using a plate reader. DPPH radical scavenging activity was calculated as the equivalent amount of Trolox corresponding to the amount of sample added, using the slope of the regression line obtained for Trolox. Whole-bean samples were tested at a solids concentration of 7.5 mg/mL, and part-specific samples (seed coat and cotyledon) at 3.0 mg/mL.

(5) Analysis of isoflavones by reversed-phase HPLC

Isoflavone profiling

To determine the proportions of 12 isoflavones in the seed coats and cotyledons of seven black bean varieties and one soybean variety, hot water extracts of these beans were analyzed by reversed-phase HPLC. The analytical conditions are shown in ***Table 2***. The concentrations of three glycosides, three aglycones, three acetylated glycosides, and three malonylated glycosides in the extracts were calculated using calibration curves based on chromatographic peak areas of mixed standard solutions of each isoflavone.

Preparation of calibration curves

Three glycosides and three aglycones were completely dissolved in dimethyl sulfoxide and ethanol, respectively, and then diluted with 0.01mol/L phosphate buffer (pH 2.6). Three acetylated glycosides and three malonylated glycosides were first dissolved in ethanol and then diluted with 0.01 mol/L phosphate buffer (pH 2.6). Each sample (20 µL) was injected into the HPLC system under the conditions shown in ***Table 2***, and retention times (RTs) were confirmed for each isoflavone. Six isoflavones (three glycosides and three aglycones) were combined and diluted 10-, 100-, and 1,000-fold with 0.01 mol/L phosphate buffer (pH 2.6) to prepare calibration standards. The acetylated and malonylated glycosides were diluted 2-, 10-, 100-, and 1,000-fold to prepare series of mixed calibration solutions. A 20-µL aliquot of each solution was injected into the HPLC and analyzed under the same conditions.

Measurement of sample isoflavone content

Sample extracts were diluted 2-fold and 4-fold with 0.01 mol/L phosphate buffer (pH 2.6). A 2-fold dilution was prepared by adding 100 µL buffer to 100 µL sample; a 4-fold dilution was prepared by adding 300 µL buffer to 100 µL sample. A 20-µL aliquot of each diluted sample was injected into the HPLC under the same conditions used to construct the calibration curves. Isoflavone contents in each sample were quantified using the respective calibration curves.

(6) Analysis of cyanidin-3-glucoside (C3G) in black beans by reversed-phase HPLC

To determine the C3G content in seed coats and cotyledons of seven black bean varieties and one soybean variety, hot water extracts were analyzed by reversed-phase HPLC. The analytical conditions are shown in ***Table 3***. The C3G concentration in each extract was calculated using a

Table 2. Isoflavones analysis HPLC conditions.

Column	Cadenza CD-C18 (75 × 84.6 mm I.D.)		
Mobile Phase A	10.0 mmol/L (sodium) Phosphate Buffer (pH 2.6)		
Mobile Phase B	Acetonitrile		
Flow Rate	1.0 mL/min		
Column Temp	40°C		
Detection	UV270 nm		
Time Program	Time (min)	FUNK	VALUE
	0	B.Conc	7
	6	B.Conc	7
	20	B.Conc	20
	30	B.Conc	20
	40	B.Conc	40
	45	B.Conc	80
	45.01	B.Conc	7
	60	B.Conc	7
	60.01	STOP	

calibration curve based on peak areas from a standard C3G solution.

Calibration curve creation

C3G was completely dissolved in purified water and then adjusted to the desired concentration with 0.01 mol/L phosphate buffer (pH 2.6). A 20-μL aliquot of each sample was injected into the HPLC system under the conditions shown in ***Table 3***, and the retention time (RT) of C3G was confirmed. The C3G solution was then diluted 10-, 100-, and 1,000-fold with 0.01 mol/L phosphate buffer (pH 2.6). Each diluted solution (20 μL) was injected and analyzed under the same conditions to construct the calibration curve.

Measurement of C3G in samples

Sample extracts were diluted 2-fold and 4-fold with 0.01 mol/L phosphate buffer (pH 2.6) as described above. A 20-μL aliquot of each diluted sample was injected into the HPLC system under the same conditions used for standard analysis. The C3G content in each sample was determined using the calibration curve.

(7) Verification of α-glucosidase inhibitory activity

α-Glucosidase inhibitory activity was measured using rat small intestinal α-glucosidase and *p*-nitrophenyl-α-D-glucopyranoside (*p*-NPG)[16]. We prepared 50 mmol/L phosphate buffer (containing 100 mmol/L NaCl, pH 7.0), 7 mmol/L *p*-NPG solution, and 0.5 mol/L Tris solution. α-Glucosidase solution was obtained by homogenizing rat small intestinal acetone powder (Sasaki Chemical Co., Kyoto) in 50 mmol/L phosphate buffer (containing 100 mmol/L NaCl, pH 7.0) and centrifuging (13,000 × g, 20 min, 4 °C) to collect the supernatant. Acarbose (3.3 mg/mL) was used as a positive control. A sample (solids concentration 7.5 mg/mL) and α-glucosidase solution were dispensed into a tube and pre-incubated at 37 °C for 5 minutes. Then, *p*-NPG (final concentration 7 mmol/L) was added and the reaction was allowed to proceed for 1 hour. The reaction was terminated by adding 0.5 mol/L Tris solution. A portion of the reaction mixture was transferred to a 96-well plate, and absorbance at 400 nm was measured to calculate α-glucosidase inhibitory activity.

(8) Statistical analysis of in vitro measurements

Data are expressed as mean ± standard deviation (SD).

The contribution rate (%) of each component to the inhibition of fluorescent AGE formation was calculated based on its content and inhibitory potency. For each component, a theoretical inhibitory contribution was estimated by multiplying its content (μg/g) by the reciprocal of its IC_{50} value ($1/IC_{50}$). The contribution rate was then expressed as the percentage of the sum of all estimated contributions, according to the following equation:

$$\text{Contibution rate (\%)} = \frac{C_i \times (1/IC_{50,i})}{\Sigma_j C_j \times (1/IC_{50,j})} \times 100$$

where C_i is the content of component i and $IC_{50,i}$ is the concentration required to inhibit 50% of fluorescent AGE formation.

(9) Effect of black bean intake on postprandial blood glucose

Subjects

Nine subjects who met the following inclusion criteria were enrolled (***Table 4***): men and women aged ≥ 20 and < 30 years at the time of consent; healthy individuals without chronic physical illness; those who received a full explanation of the purpose and content of the study, were competent to consent, fully understood the study, and voluntarily provided written informed consent; those able to attend the designated study dates; and those deemed suitable for participation by the principal investigator.

Table 3. C3G analysis conditions.

Column	Cadenza CD-C18 (75 × 84.6 mm I.D.)		
Mobile Phase A	10.0 mmol/L (sodium) Phosphate Buffer (pH 2.6)		
Mobile Phase B	Acetonitrile		
Flow Rate	1.0 mL/min		
Column Temp	40 °C		
Detection	UV510 nm		
Time Program	Time (min)	FUNK	VALUE
	0.01	B.Conc	7
	6.00	B.Conc	7
	20.00	B.Conc	20
	20.01	B.Conc	80
	25.00	B.Conc	80
	25.01	B.Conc	7
	35.00	B.Conc	7
	35.01	STOP	

Table 4. Subjects profile.

	Unit	Total	Male	Female
Number of subjects		9	1	8
Age	years	22.8 ± 1.2	23	22.8 ± 1.3
Body height	cm	161.2 ± 7.8	170.8	160.0 ± 7.4
Body weight	kg	52.3 ± 7.3	60.5	51.3 ± 7.0
BMI		20.1 ± 2.3	20.7	20.0 ± 2.5

Results are expressed as mean ± standard deviation. BMI, body mass index.

Table 5. Result of the blood chemistry test.

test item	Unit	Measured value	Reference range
FBG	mg/dL	78.4 ± 5.0	70 - 109
HbA1c	%	5.1 ± 0.1	4.6 - 6.2
IRI	μU/mL	5.5 ± 2.2	1.7 - 10.4
Total cholesterol	mg/dL	191.0 ± 44.4	120 - 219
TG	mg/dL	76.9 ± 31.8	30 - 149
HDL-C	mg/dL	78.1 ± 11.8	40 - 85
LDL-C	mg/dL	103.6 ± 40.6	65 - 139

Results are expressed as mean ± standard deviation, n = 9, FBG, fasting blood glucose; IRI, immunoreactive insulin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TG, triglyceride; AST, aspartate transaminase; ALT, alanine transaminase; γ-GT, γ-glutamyltransferase.

Survey items and test procedures

Subjects completed a questionnaire on demographic characteristics, medical history, and food allergy status, and underwent blood testing (***Table 5***). Glucose concentrations in interstitial fluid were measured using a FreeStyle Libre Pro device (Abbott Laboratories, Chicago, USA) every 15 minutes during the study period, and blood glucose levels were recorded [17].

Study protocol

As in previous reports [18-22]. This study was conducted according to the unified protocol of the Japan Glycemic Index (GI) Study Group [23]. Subjects were instructed to comply with the following during the study period: avoid irregular lifestyle patterns such as sleep deprivation and overeating; maintain the same quantity and quality of diet, physical activity, and sleep as before enrollment; refrain from starting new health foods or supplements; and avoid other behaviors that could affect study results.

On the day before and the day of testing, subjects were instructed to: avoid strenuous exercise on the day before testing; sleep at least 6 hours on the night before testing; abstain from alcohol from the day before testing until completion of the test; avoid high-fat foods at dinner the day before testing; and consume only water after 22:00. On the test day, subjects were instructed to avoid exercise and physical activity that could cause sweating until the test was completed. For female participants, tests were not conducted during menstruation. During testing, subjects sat quietly at rest and were prohibited from making phone calls, sleeping, engaging in intense mental activities (*e.g.*, email, computer use), or performing physical activity. After intake of the test food, subjects remained fasting until the end of the test.

Subjects attached the Libre Pro sensor to the outer upper arm at least two days before the test. There were no restrictions on bathing, swimming, or exercise while wearing the sensor. Testing began at 10:00; subjects consumed the test meal within 10 minutes. Thereafter, they remained seated and watched a video in a relaxed state until the end of the session at 12:00.

Each mouthful of the test food was chewed at least 30 times before swallowing. Blood glucose levels were evaluated at baseline (before ingestion; first measurement) and at 15 (second), 30 (third), 45 (fourth), 60 (fifth), 90 (sixth), and 120 (seventh) minutes after the start of meal ingestion.

Test foods

The nutritional composition of the test foods was calculated from values listed on the respective product labels, and the carbohydrate intake per serving was standardized to 50 g (***Table 6***). Commercially available packaged cooked rice, furikake (seasoning topping), and black bean products were used. Packaged cooked rice was "Sato's Rice, Niigata Koshihikari 150 g" (Sato Foods Industry, Niigata, Japan). Furikake was "Nori Tama" (Marumiya Foods Industry, Tokyo, Japan). Two black bean products were used: "Roasted Black Beans (Tanba Grape Black Beans)" (Kitao Shoji Co., Ltd., Kyoto) and "Crispy Black Bean Snacks (Kurosengoku)" (Kurosengoku Business Cooperative, Hokkaido, Japan).

The test meals were labeled A, B, and C and were composed as follows:

A (reference meal): 150 g packaged cooked rice + 2.5 g furikake (total carbohydrate: 50 g)
B (test meal): 138 g packaged cooked rice + Tanba Grape Black Beans (total carbohydrate: 50 g)

Table 6. Nutrition facts of test food.

Test food	Serving unit (g)	Energy (kcal)	Protein (g)	Fat (g)	Carbohydrates (g)
A	152	212	3	0	50
B	158	272.8	10.9	3.1	50
C	153.4	258.7	8.6	3.1	50

A, rice 150 g + seasoning 2.5 g; B, rice 138 g + Tanbabudoukuromame 20 g; C, rice 138 g + Kurosenngoku 15.4 g.

C (test meal): 138 g packaged cooked rice + Kurosengoku (total carbohydrate: 50 g)

All test meals (A, B, and C) were consumed within 10 minutes of the start of testing.

Selection of subjects for safety analysis

Subjects who consumed any of the test meals at least once were included in the safety analysis set.

Selection of subjects for efficacy analysis

Subjects who completed the full study schedule and all study procedures were eligible for efficacy analysis. Those who exhibited behaviors that significantly compromised the reliability of test results, or who were found to meet exclusion criteria or failed to comply with restrictions after starting intake, were excluded.

Statistical analysis (clinical study)

Safety evaluations were performed on the safety analysis set, and adverse events and side effects were assessed by summarizing symptom type, severity, and frequency. Efficacy analyses were conducted on the efficacy analysis set.

Changes in blood glucose (ΔBG) were calculated by subtracting the pre-ingestion value (0-minute baseline) from each subsequent time-point value. The maximum change in blood glucose within 120 minutes after test start was defined as ΔCmax (maximum blood glucose change). The incremental area under the blood glucose curve (iAUC) was calculated according to the unified protocol of the Japan GI Study Group[23].

Statistical analyses were performed using BellCurve for Excel (Social Information Services, Shinjuku, Tokyo). Blood glucose values are expressed as mean ± standard error (SE). Between-group comparisons were performed using the Bonferroni multiple comparison test. A two-sided p-value < 0.05 was considered statistically significant (*), and $0.05 \leq p < 0.10$ was considered to indicate a trend (†).

Ethical standards

This study was conducted in accordance with the Ethical Guidelines for Medical Research Involving Human Subjects (issued jointly by the Ministry of Education, Culture, Sports, Science and Technology and the Ministry of Health, Labour and Welfare, Japan). Study procedures were fully explained to the subjects in advance, and written informed consent was obtained from all participants. The study was approved by the Ethics Review Committee for "Research Involving Human Subjects" of the Society of Glycative Stress Research, which examined both ethical and scientific validity (approval number: GSE22003).

Results

Fluorescent AGE formation inhibitory effect of black beans

All seven black bean varieties exhibited inhibition rates of ≥ 15%, with an overall mean of 55.5 ± 23.31% (mean ± SD, n = 7, ***Fig. 1***). Kurosengoku showed the highest inhibition of fluorescent AGE formation (85.3 ± 2.5%, n = 3), whereas Tanba Black Beans (Bicchu) Kuronowara showed the lowest (15.7 ± 0.3%, n = 3). Significant differences in fluorescent AGE formation inhibition rates were observed among all varieties except between Tanba Black Beans (Bicchu) Kuronowara and Tanba Black Beans (Hikiri) ($p < 0.001$). When IC_{50} values (the concentration required to inhibit fluorescent AGE formation by 50%) were calculated, they were lowest in the following order: Kurosengoku (0.122 mg/mL) < Tanba Black Beans (Tobikiri) (0.267 mg/mL) < Hikarikurodaizu (0.702 mg/mL) < Hakodate Black Beans (0.886 mg/mL) < Tanba Black Beans (Bicchu) Kuronowarai (0.892 mg/mL) < Gankuimame (0.894 mg/mL) < Tanba Grape Black Beans (0.943 mg/mL). For comparison, the IC_{50} of yellow soybeans was 2.61 mg/mL, and that of the positive control AG was 0.068 mg/mL.

Fluorescent AGE inhibition by bean part

All seed coat extracts exhibited inhibitory effects, with a mean of 92.6 ± 4.18% (mean ± SD, n = 7, ***Fig. 2***). Gankuimame seed coat showed the strongest inhibition (99.7 ± 0.28%, n = 3), while Hakodate Kuromame showed the weakest (87.3 ± 0.68%, n = 3). In cotyledons, all seven varieties also showed inhibitory effects.

The average inhibition rate was 49.8 ± 16.5% (n = 7). Kurosengoku cotyledons showed the highest inhibition (58.5 ± 1.05%, n = 3), while Tanba Grape Black Beans showed the lowest. (9.51 ± 0.93%, n = 3). A t-test comparing seed coats (n = 7) and cotyledons (n = 7) showed a significant difference ($p < 0.01$). For comparison, yellow soybeans exhibited 5.1 ± 5.1% inhibition in cotyledons and 30.7 ± 3.2% in seed coats.

Inhibitory effects of 12 isoflavones and C3G

Three glycosides and three aglycones of isoflavones were found to inhibit the formation of fluorescent AGEs. The IC_{50} values were lowest in the following order: glycitein (5.88 ng/mL) > glycitin (189 ng/mL) > daidzein (7.01×10^3 ng/mL) > daidzin (8.75×10^3 ng/mL) > genistin (20.3×10^3 ng/mL) > genistein (1.02×10^6 ng/mL), indicating a greater inhibitory effect on fluorescent AGE formation. In the two pairs of daidzin and daidzein, and glycitin and glycitein, the aglycones had a greater anti-glycation effect than the

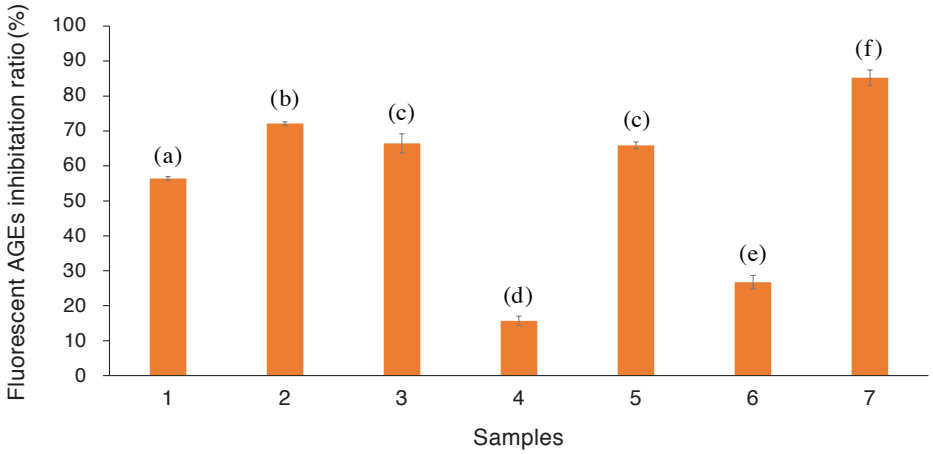

Fig. 1. **Inhibitory effect of Black beans (whole) on AGEs and intermediate formation in the HSA-Glucose reaction model.**

Results are expressed as mean ± SD. Black beans were introduced into glycation models containing 40 mg/mL HSA and 2.0 mol/L glucose (n = 3). After 40-hour incubation at 60°C, fluorescent AGEs were measured by Ex 370 nm / Em 440 nm. AGEs, advanced glycation endoproducts; HSA, human serum albumin; SD, standard deviation.

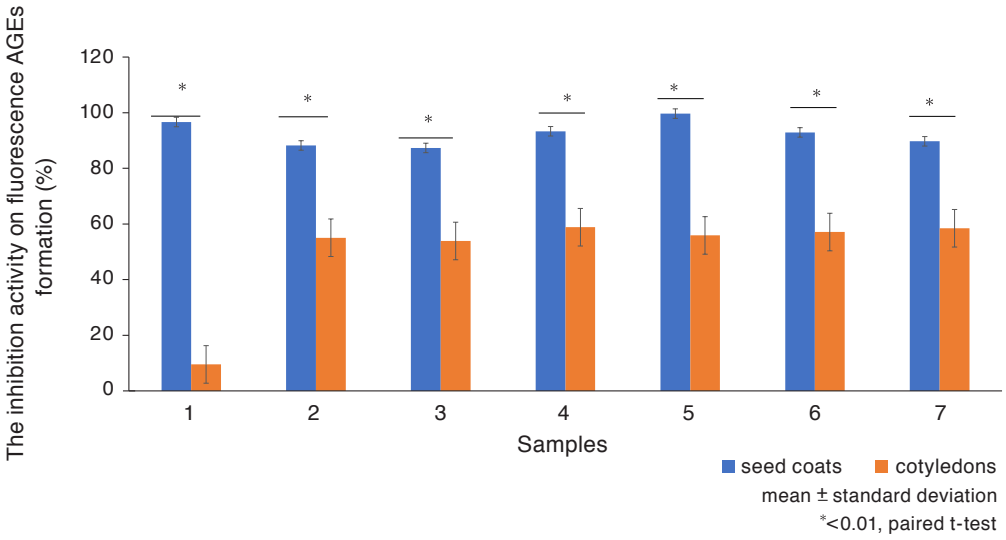

Fig. 2. **Inhibitory effect of black beans (by part) on AGEs and intermediate formation in the HSA-Glucose reaction model.**

Results are expressed as mean ± SD, *p < 0.01, paired t test, n = 3. Black beans were introduced into glycation models containing 40 mg/mL HSA and 2.0 mol/L glucose. After 40-hour incubation at 60°C, fluorescent AGEs were measured by Ex 370 nm/Em 440 nm. AGEs, advanced glycation endoproducts; HSA, human serum albumin; SD, standard deviation.

glycosides, while for genistin and genistein, the glycoside showed greater anti-glycation activity than the aglycone. Malonylated glycosides and acetylated glycosides did not exhibit any inhibitory effect on fluorescent AGE formation. The IC_{50} of C3G was 10.7 µg/mL, indicating a greater antioxidant effect than genistin and genistein.

DPPH radical scavenging activity

All seven black bean varieties exhibited DPPH radical scavenging activity, with a mean value of 141 ± 47.4 µmol Trolox Eq/L (mean ± SD, n = 7, ***Fig.3***). Among the varieties, Kurosengoku showed the highest activity (247 µmol Trolox Eq/L), while Hikarikurodaizu showed the lowest (96.7 µmol Trolox Eq/L). For comparison, yellow soybeans showed a DPPH radical scavenging activity of 128 µmol Trolox Eq/L. The DPPH radical scavenging activity of seed coats and cotyledons of the seven black bean varieties is shown in ***Fig. 4***. In black beans, seed coats exhibited higher activity than cotyledons; in yellow soybeans, seed coats also showed higher activity than cotyledons. The mean DPPH radical scavenging activity in seed coats was 369 ± 131 µmol Trolox Eq/L (n = 7). Kurosengoku exhibited the highest activity (550 µmol Trolox Eq/L), and Tanba Black Beans (Bicchu) Kuronowara the lowest (154 µmol Trolox Eq/L). The mean DPPH radical scavenging activity in cotyledons was 244 ± 104 µmol Trolox Eq/L (n = 7). Tanba Grape Black Beans showed the highest activity (336 µmol Trolox Eq/L), and Tanba Black Beans (Bicchu) Kuronowara the lowest (11.8 µmol

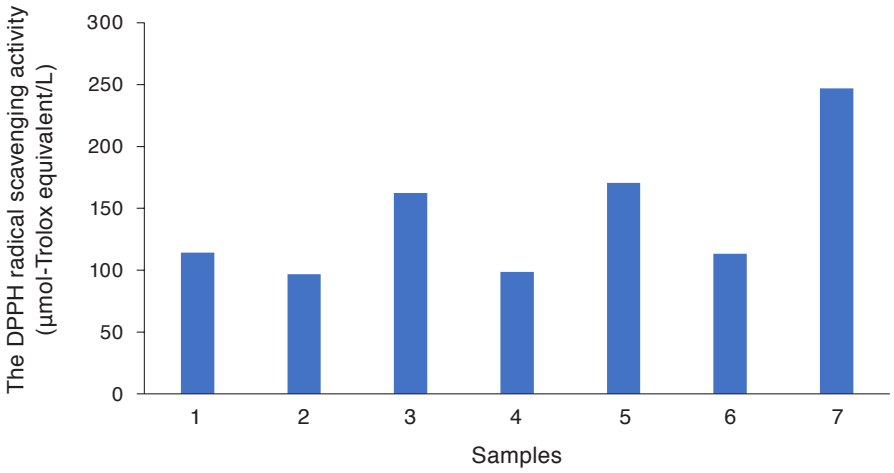

Fig. 3. **Anti-oxidative activity of black beans (whole).**

DPPH radical scavenging activity of black bean was measured by a method of calculating the equivalent amount of Trolox using it as a standard substance (n = 2). DPPH, 1,1-diphenyl-2-picrylhydrazyl.

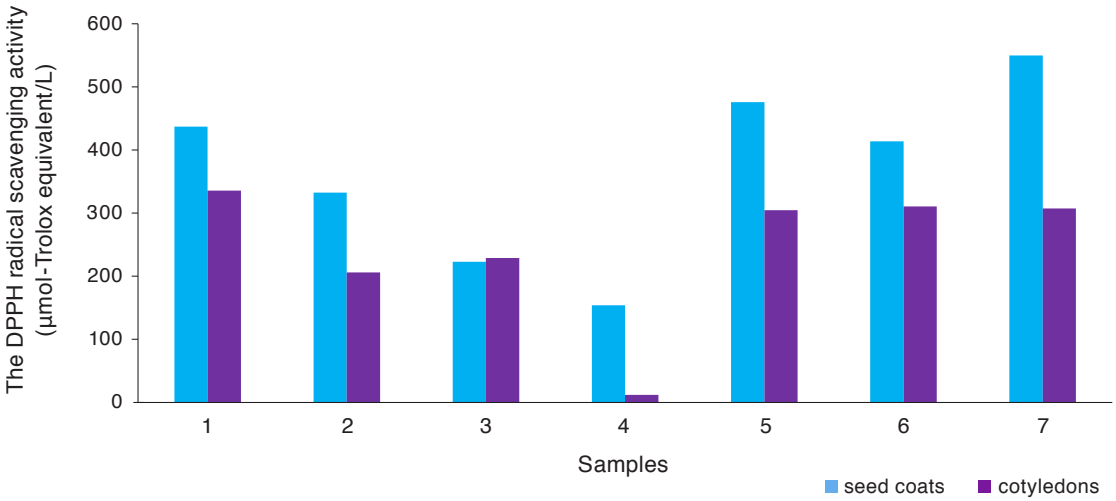

Fig. 4. **Anti-oxidative activity of black beans (by part).**

DPPH radical scavenging activity of black bean was measured by a method of calculating the equivalent amount of Trolox using it as a standard substance (n = 2). DPPH, 1,1-diphenyl-2-picrylhydrazyl.

Trolox Eq/L). For comparison, DPPH radical scavenging activity in yellow soybeans was 143 μmol Trolox Eq/L in the seed coat and 501 μmol Trolox Eq/L in the cotyledons.

When 6 isoflavones and C3G were tested at a solid concentration of 0.1 mg/mL, C3G and the isoflavones genistein and genistin showed notable activity. The DPPH radical scavenging activities were 1,080 μmol Trolox Eq/L for C3G, 137 μmol Trolox Eq/L for genistein, and 33.1 μmol Trolox Eq/L for genistin.

Analysis of isoflavones and C3G in black beans by reversed-phase HPLC

The retention times (RTs) for the 12 isoflavones were as follows (mean ± SD, n = 3): daidzin, 17.8 ± 0.03 min; glycitin, 18.9 ± 0.02 min; genistin, 21.8 ± 0.03 min; malonyldaidzin, 22.7 ± 0.04 min; malonylglycitin, 23.1 ± 0.04 min; acetylglycitin, 25.4 ± 0.05 min; acetyldaidzin, 26.0 ± 0.20 min; malonylgenistin, 26.2 ± 0.04 min; daidzein, 28.3 ± 0.04 min; glycitein, 30.7 ± 0.07 min; acetylgenistin, 32.5 ± 0.13 min; and genistein, 38.4 ± 0.02 min. Isoflavone contents in seed coats and cotyledons of the seven black bean varieties and one soybean variety, calculated using the calibration curves, are shown in ***Fig. 5***.

The RT of C3G was 13.0 ± 0.0 min (n = 3). C3G was not detected in the cotyledons of any of the seven black bean varieties nor in either the seed coat or cotyledons of yellow soybeans. In the seed coat of Tanba Black Beans (Tobikiri), C3G levels were at the lower limit of detection. The amounts of C3G in the seed coats of the seven black bean varieties calculated from the calibration curve are shown in ***Fig. 6***.

α-Glucosidase inhibitory activity

Fig. 7 shows the α-glucosidase inhibitory activity of hot water extracts of black beans and that of acarbose (final

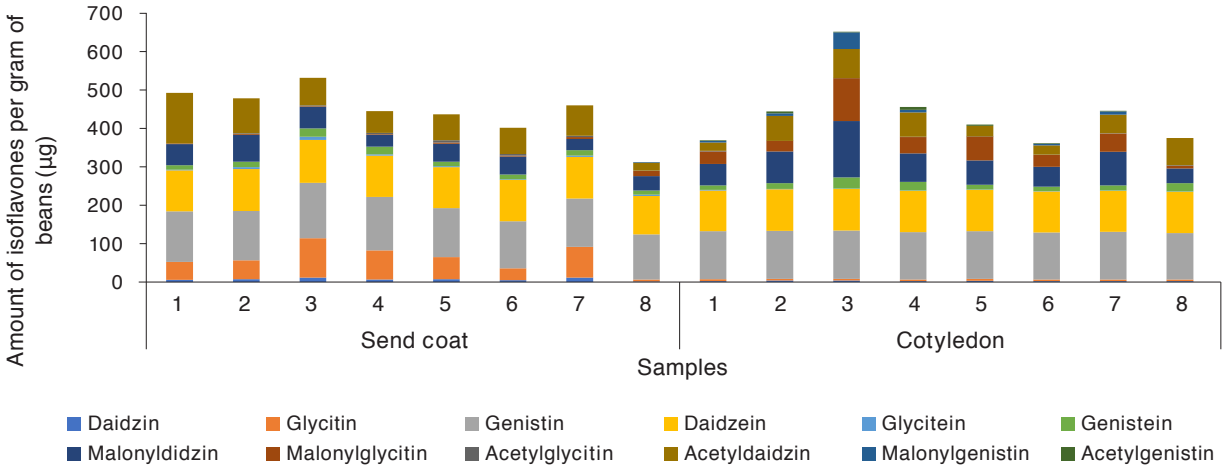

Fig. 5. **Isoflavone content per gram of beans determined by reversed-phase HPLC.**
Values are expressed as μg/g of beans.

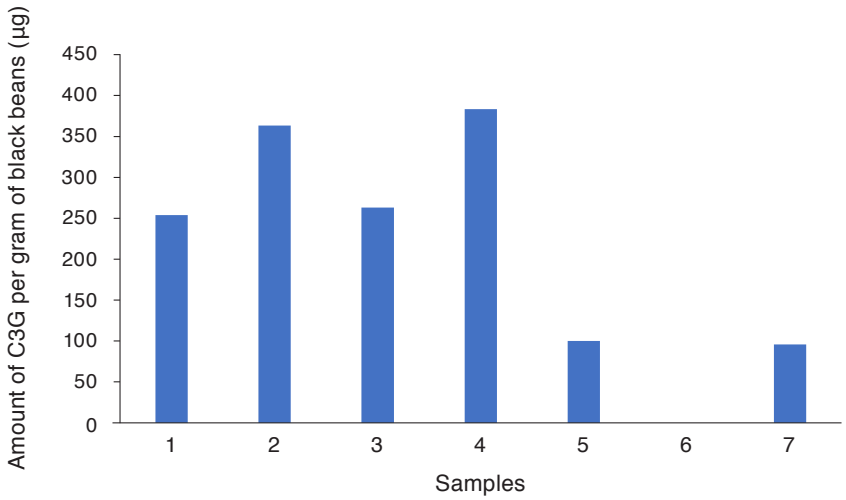

Fig. 6. **C3G content per gram of black beans determined by reversed-phase HPLC.**
Values are expressed as μg/g of beans. C3G: cyanidin-3-glucoside.

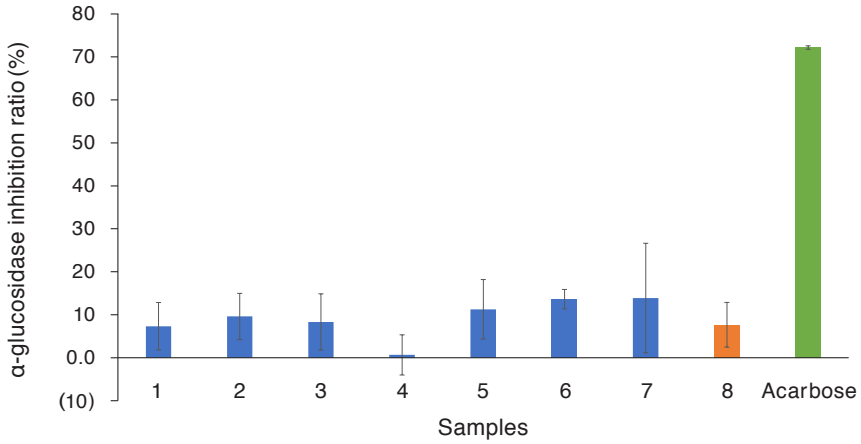

Fig. 7. **α-Glucosidase inhibitory activity of black bean extracts.**
Samples were reacted with 0.9 U/mL α-glucosidase and 7 mmol/L p-nitrophenyl-α-D-glucopyranoside (*p*-NPG) at 37°C for 1 h. Absorbance was measured at 400 nm (final concentrations: black bean extracts 0.225 mg/mL; acarbose 0.099 mg/mL, n = 2).

concentration: 0.099 mg/mL), used as a positive control. At a final bean concentration of 0.225 mg/mL, all varieties showed similar, relatively weak α-glucosidase inhibitory activity, with no significant differences observed among varieties.

Effect of black beans on postprandial blood glucose

Safety evaluation

No adverse events were reported during the study.

Efficacy evaluation

No subjects met the exclusion criteria; therefore, all nine participants were included in the efficacy analysis.

Efficacy analysis

All nine subjects (one male, eight females; age 22.8 ± 1.2 years, height 161.2 ± 7.8 cm, weight 52.3 ± 7.3 kg, BMI 20.1 ± 2.3 kg/m^2; mean ± SD) completed the study. ***Fig. 8*** shows the time course of ΔBG after ingestion of each test meal. Fasting blood glucose levels at baseline (0 min) ranged from 78.2 to 85.2 mg/dL. Blood glucose levels increased after ingestion of each test meal, peaked at 45 minutes, and then decreased until 120 minutes.

After ingestion of test meal A, ΔBG at 120 minutes (Δ120 min) was significantly higher than that after test meal B ($p < 0.05$) and tended to be higher than that after test meal C at 60 and 120 minutes ($p < 0.10$). ΔCmax values were as follows: A, 63 ± 6 mg/dL; B, 61 ± 6 mg/dL; and C, 58 ± 7 mg/dL (mean ± SE, n = 9, ***Fig. 9-a***). Compared with A, ΔCmax was 2.2 mg/dL (3.5 %) lower for B and 5.1 mg/dL (8.1 %) lower for C.

The iAUC values were 4,490 ± 630 mg/dL・min for A, 4,040 ± 602 mg/dL・min for B, and 3,360 ± 562 mg/dL・min for C (n = 9, ***Fig. 9-b***). Compared with A, iAUC was 447 mg/dL・min (10.0 %) lower for B and 1,460 mg/dL・min (32.6 %) lower for C.

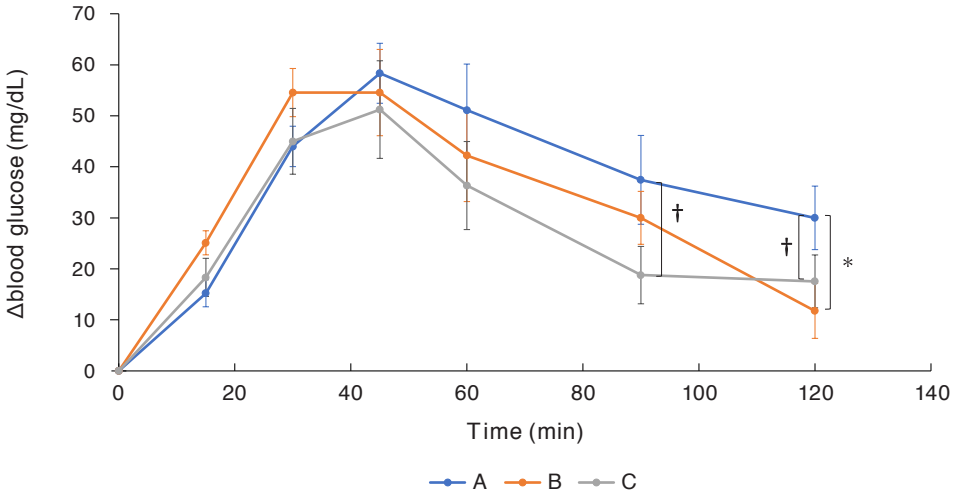

Fig. 8. **Changes in ΔBG after ingestion of test meals.**
Values are mean ± SE (n = 9). † $0.05 \leqq p < 0.1$, * $p < 0.05$ vs. standard meal (A), Bonferroni test.
Test meal composition is shown in **Table 6**. ΔBG, changes in blood glucose; SE, standard error.

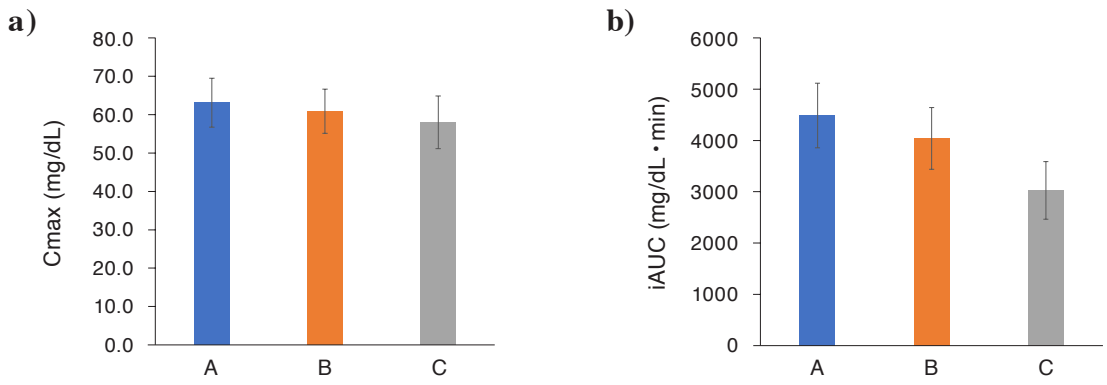

Fig. 9. **ΔCmax (a) and iAUC (b) after ingestion of test meals.**
Values are mean ± SE (n = 9). Test meal composition is shown in **Table 6**. iAUC: incremental area under the curve of ΔBG; ΔBG, changes in blood glucose; ΔCmax, maximum blood glucose level change; SE, standard error.

Discussion

Previous research on black bean components

Black beans (black soybeans) are nutrient-dense and contain numerous functional components with potential health benefits. In addition to nutrients common to yellow soybeans, they are characterized by bioactive components derived from the black seed coat. Anthocyanins (black soybean polyphenols) are pigments in the black seed coat with strong antioxidant properties and are also found in blueberries. Soy isoflavones, as in yellow soybeans, exhibit estrogen-like effects. Other nutrients include soy protein, dietary fiber, oligosaccharides, glycoside saponins, minerals (iron, potassium, calcium, magnesium), and vitamins (E, K, and B-complex). Lecithin is a major phospholipid component of cell membranes, and choline is a precursor of neurotransmitters.

Previous studies on black beans are summarized below.

Health functions (cardiovascular, metabolic, bone, and quality of life)

Black beans have repeatedly been reported to exert protective effects on the cardiovascular and metabolic systems. In a randomized controlled trial of patients with prehypertension to stage I hypertension, intake of black bean-derived peptides significantly decreased systolic and diastolic blood pressure and improved oxidative stress markers[24]. In animal experiments using spontaneously hypertensive rats and high-fat diet-fed rats, long-term administration of black bean extract or black bean extract powder reduced blood pressure, improved lipid metabolism, and suppressed fatty acid accumulation in subcutaneous adipose tissue[25-28].

Regarding insulin resistance and glucose metabolism, black bean anthocyanins have been suggested to improve insulin resistance by regulating adipocyte differentiation and adiponectin expression, and by suppressing reactive oxygen species[29-31]. For the bone and musculoskeletal system, a bone-strengthening supplement containing black beans together with calcium and magnesium has been reported to improve femoral trabecular bone mass and bone mineral density in osteoporosis model mice[32]. Black bean tea and other foods containing soy isoflavones have been shown to reduce bone resorption markers and may contribute to the prevention of osteoporosis in menopausal women[33].

Furthermore, a long-term intake study of black bean broth reported reductions in body fat and blood pressure, along with improvements in subjective QOL indicators such as bowel movements, sleep, and fatigue[34]. Intake of black bean natto has also been reported to be associated with improvements in menopausal complaints and mood status [35,36]. Together, these findings suggest that black beans exert multifaceted health functions on the cardiovascular system, metabolism, bone, and QOL.

Functional components and mechanisms (anthocyanins, isoflavones, etc.)

The functionality of black beans is thought to arise from the anthocyanins abundant in the seed coat, the isoflavones present in the cotyledons, and various polyphenols and glycosides such as saponins[37-39]. Analyses of black bean tea infusions have shown that isoflavone and anthocyanin contents vary markedly depending on infusion conditions, and that some products with added soy isoflavones have particularly high levels[37]. Saponins, including the soyasaponin group, have been isolated and quantified from various soybeans, including black beans[38], and are suggested to contribute to antioxidant effects and regulation of lipid metabolism.

At the cellular level, black bean seed coat extract has been reported to inhibit hydrogen peroxide-induced cell death in liver-derived HepG2 cells and to exert antioxidant and cytoprotective effects via modulation of ERK signaling and increased total protein phosphatase activity[40]. In human gastric epithelial AGS cells, black bean anthocyanins exhibit anti-inflammatory effects by suppressing Helicobacter pylori-induced reactive oxygen species generation, NF-κB activation, and inflammatory gene expression, and by decreasing IL-8 production[41]. In adipocyte models, black bean anthocyanins affect 3T3-L1 cell differentiation, insulin signaling, and GLUT4 expression, suggesting a potential to improve insulin resistance[29-31].

With respect to thrombosis and vascular function, black bean anthocyanins – particularly cyanidin 3-O-glucoside – have been shown to exert antithrombotic effects in vascular endothelial cells[42,43]. Isoflavones ingested in the form of black bean tea have also been reported to exert differential effects on flow-mediated vasodilation and arterial stiffness in pre- and postmenopausal women, depending on smoking status[44]. A beverage containing fermented black beans has been shown to suppress lipid peroxidation and maintain antioxidant enzyme activities in a rat model of renal oxidative damage induced by ferric nitrilotriacetate[45]. Furthermore, black beans fermented with Aspergillus awamori have shown enhanced antiproliferative activity against tumor cells and stronger Cu^{2+}-chelating capacity than non-fermented black beans[46,47].

In addition, ethanol extracts of herbal medicines included in Kampo formulations containing black beans have been reported to modulate immune and inflammatory responses, such as by suppressing cytokine-induced thyroid cell damage and abnormal MHC class II expression[48,49].

Food uses (tea, fermented beverages, natto)

Black beans are consumed in a wide range of forms, including traditional dishes such as simmered beans and broth, as well as tea beverages, fermented beverages, fermented soybean foods, desserts, and health supplements. Numerous black bean teas and beverages containing black bean extracts are commercially available, and their infusion conditions and isoflavone/anthocyanin contents have been extensively investigated[37,50]. Citric acid beverages and vinegars combining black bean polyphenols with rice-koji-derived citric acid have been reported to show beneficial effects on blood lipids and liver function indices in growing rats, and their manufacturing conditions have been optimized [51-53]. Intervention studies using citric acid beverages containing black bean extracts have also evaluated health-promoting effects and subjective improvements in physical condition in middle-aged and older adults[54].

As fermented foods, black bean natto has been used in intervention studies aimed at alleviating menopausal

symptoms and improving mood[35,36]. Lactic acid-fermented black bean products have shown antioxidant properties and constipation-alleviating effects in mouse experiments[55]. Health drinks and supplements using black bean extracts or black bean peptides have been evaluated in randomized controlled trials and animal experiments for effects on blood pressure, oxidative stress, and kidney function, as noted earlier[24,26,45].

Furthermore, recipes such as main dishes combining black beans with small fish and desserts like black bean agar, which are designed with consideration for blood glucose levels, have been proposed for osteoporosis prevention and diabetic diets[56,57]. Other studies have examined the health effects of daily consumption of black bean broth[34], evaluated the antiviral activity of hot water extracts of black beans against respiratory viruses and their ability to promote airway ciliary motion[58], and investigated the contribution of cooking black beans in iron pots to dietary iron intake[59]. Thus, the functionality of black beans has been evaluated across a broad spectrum of applications, from beverages and daily dishes to functional foods.

Safety and risks

Black beans have traditionally been consumed as a food and are generally regarded as highly safe. However, recent case reports and toxicology studies have highlighted certain risks associated with excessive intake or specific susceptible individuals. In a 28-day repeated-dose toxicity study in rats, feeding diets containing up to 5% black bean hot water extract caused no adverse changes in general condition, body weight, hematology, clinical chemistry, organ weight, or histopathology. The no-observed-adverse-effect level (NOAEL) was estimated at 3,618 mg/kg/day in males and 4,066 mg/kg/day in females[60,61]. Conversely, there are also reports that black beans can reduce the acute toxicity of Aconiti tuber when co-administered, suggesting a role as a safety-modulating factor in Kampo formulations[62].

Three human cases of anaphylaxis have been reported following consumption of health drinks and foods containing soy protein and teabag-type black bean tea, with links to specific soy allergen components such as Gly m 3 and Gly m 4[63]. In another report of food-dependent exercise-induced anaphylaxis after consumption of yuba (bean curd skin), skin prick tests were positive for multiple soy foods including black beans, indicating that caution is warranted when soy-allergic individuals consume black beans[64].

A case of suspected iron overload has been reported in an elderly woman who consumed large quantities of black beans over a prolonged period[65]. When black beans are combined with iron-fortified dishes or prepared in iron pots, attention should therefore be paid to total iron intake[59]. In addition, a case of esophageal burn-type ulcer caused by ingestion of extremely hot black bean broth has been reported[66], highlighting the importance of temperature control during cooking and consumption.

Isoflavones in black bean tea are generally considered safe at typical intake levels, but some black bean tea products with added soy isoflavones have very high isoflavone contents, and caution is needed regarding long-term excessive intake[33,37]. Meanwhile, herbal extracts containing black beans have been reported to exert protective effects on immune and inflammatory responses, such as suppressing cytokine-induced thyroid cytotoxicity and abnormal MHC class II expression[48].

Overall, black beans are considered highly safe when consumed in appropriate amounts. However, individuals with allergies or certain underlying conditions should pay attention to intake amount, cooking methods, and the isoflavone content of specific products.

Findings of this study

This study demonstrated that hot water extracts of seven commercially available black bean varieties inhibit fluorescent AGE formation and scavenge DPPH radicals in an HSA–glucose system in a concentration-dependent manner, with the seed coat being the primary locus of activity, particularly due to C3G and specific isoflavones. Significant inter-varietal differences in anti-glycation activity were also observed, including marked activity in Kurosengoku. In addition, when black beans were added to rice, both ΔCmax and iAUC for postprandial blood glucose were reduced compared with white rice alone. These findings indicate that black beans are a food that can alleviate glycative stress by combining anti-glycation and antioxidant properties with suppression of postprandial blood glucose elevation.

Inhibitory effect of black beans on fluorescent AGE formation

All seven black bean varieties exhibited inhibitory effects on fluorescent AGE formation. Fluorescent AGEs are detected at an excitation wavelength of 370 nm and an emission wavelength of 440 nm, and are characterized by yellowish-brown fluorescence[67]. Representative fluorescent AGEs include crosslines (Ex 379 nm/Em 463 nm) and pyropyridines (Ex 370 nm/Em 455 nm). Crosslines have protein-crosslinking properties, and the formation of AGEs in proteins leads to reduced tissue elasticity and flexibility[67].

In this study, using an HSA glycation model, fluorescence detected under the above conditions was considered to be derived from fluorescent AGEs, and inhibition rates were calculated. All seven black bean varieties and yellow soybeans exhibited inhibitory effects, with the seven black bean varieties having significantly greater effects than yellow soybeans. This suggests that black beans are more effective than yellow soybeans in inhibiting fluorescent AGE formation, thereby alleviating glycative stress and potentially preventing protein-derived AGE formation *in vivo*.

We also examined the contributions of seed coats and cotyledons to the inhibition of fluorescent AGE formation in the HSA–glucose model. Seed coats of black beans showed stronger inhibitory effects than cotyledons. However, because cotyledons account for a larger proportion of the bean mass, calculations of their relative contributions indicated that cotyledons contributed more to total inhibition than seed coats at the whole-bean level.

Plant materials with strong anti-glycation properties have been reported previously, including yacon (*Smallanthus sonchifolius*), red rhubarb vegetables, apples, and mangosteen. For many of these, as in black beans, the peel or skin has stronger effects than the edible portion[68].

We further calculated the contribution of 6 isoflavones (3 glycosides and 3 aglycones) and C3G to the inhibition of fluorescent AGE formation by HSA relative to black bean part (seed coat or cotyledon). The results (***Table 7***) showed that in seed coats, glycitin had the highest contribution (83.5 ± 6.0 %), whereas in cotyledons, daidzein had the highest contribution (72.6 ± 3.0 %). The contribution of C3G, which was detected only in seed coats, was 44.9 ± 9.1%. These findings suggest that C3G is one of the key reasons why seed coats demonstrate stronger inhibitory effects than cotyledons.

Glycative stress-alleviating effects of black beans

Alleviating glycative stress can be achieved by suppressing postprandial hyperglycemia, inhibiting glycation reactions, and promoting the degradation and excretion of glycation products[8].

In this study, black beans showed only weak α-glucosidase inhibitory activity, suggesting that inhibition of α-glucosidase plays a minor role in their effect on postprandial hyperglycemia. Previous studies have reported that procyanidin-rich fractions derived from black bean seed coats and related compounds suppress hyperglycemia and obesity[29]. It has also been suggested that a part of the mechanism by which procyanidins suppress hyperglycemia involves incretin-like activity, in which they promote secretion of the gastrointestinal hormone GLP-1 from intestinal L cells, thereby enhancing insulin secretion[29].

In light of these findings, the mechanism by which black beans suppressed postprandial blood glucose in the present study—despite their weak α-glucosidase inhibitory activity—may involve procyanidins contained in black beans exerting incretin-like effects that promote glucose-stimulated insulin secretion from pancreatic β-cells.

Our results suggest that black beans inhibit the formation of AGEs, demonstrating that black beans alleviate glycative stress both *in vitro* and in a semi-*in vivo* setting. Whether black beans also facilitate the degradation of AGEs requires further investigation.

Conclusion

This study demonstrated that black beans, especially their seed coats rich in C3G and isoflavones, inhibit fluorescent AGE formation and exert antioxidant effects. The suppression of postprandial blood glucose suggests that black beans are a practical dietary strategy for reducing glycative stress.

Conflict of Interest Declaration

There are no conflicts of interest regarding the conduct of this study.

Table 7. Contribution rate (%).

	Sample No.	Daidzin	Glycitin	Genistin	Daizein	Glysitein	Genisitein	C3G
Sead coat	1	9.9	82.2	31.3	43.3	0.0	22.9	53.2
	2	8.9	82.9	29.8	44.6	0.0	23.3	57.2
	3	3.9	79.2	20.8	37.9	0.0	19.4	59.2
	4	7.5	88.7	29.6	42.6	0.0	25.2	58.8
	5	7.7	75.2	25.7	38.9	0.0	19.0	43.8
	6	10.6	81.0	33.6	46.4	0.0	25.4	0.0
	7	14.6	94.9	30.6	44.9	0.0	23.9	48.2
Cotyledon	1	0.0	25.1	60.7	128.8	0.0	44.1	0.0
	2	0.0	36.9	39.4	65.9	0.0	31.7	0.0
	3	0.0	17.4	32.5	61.7	0.0	36.0	0.0
	4	0.0	29.5	34.7	60.3	0.0	33.8	0.0
	5	0.0	39.1	40.2	65.8	0.0	30.7	0.0
	6	0.0	32.4	37.2	63.1	0.0	27.5	0.0
	7	0.0	34.7	37.7	62.4	0.0	29.0	0.0

Contribution rates were calculated from the content of each component and its IC_{50} value for inhibition of fluorescent AGE formation.

Reference

1) Negre-Salvayre A, Salvayre R, Augé N, et al. Hyperglycemia and glycation in diabetic complications. *Antioxid Redox Signal*. 2009; 11: 3071-3109.
2) Yonei Y, Yagi M, Sato K, et al. Glycative stress: Molecular impacts and defense mechanisms. *Glycative Stress Res*. 2023; 10: 145-158.
3) Yonei Y, Saito Y, Yagi M, et al. From fatty liver to steatohepatitis: Involvement of aldehydes. *Glycative Stress Res*. 11: 79-93, 2024.
4) Yagi M, Takabe W, Wickramasinghe U, et al. Effect of heat-moisture-treated high-amylose corn starch-containing food on postprandial blood glucose. *Glycative Stress Res*. 2018; 5: 151-162.
5) Nagai R, Mori T, Yamamoto Y, et al. Significance of advanced glycation end products in aging-related disease. *Anti-Aging Med*. 2010; 7: 112-119.
6) Reiser KM. Nonenzymatic glycation of collagen in aging and diabetes. *Proc Soc Exp Biol Med*. 1998; 218: 23-37.
7) Saito M, Fujii K, Soshi S, et al. Reductions in degree of mineralization and enzymatic collagen cross-links and increases in glycation induced pentosidine in the femoral neck cortex in cases of femoral neck fracture. *Osteoporos Int*. 2006; 17: 986-995.
8) Reddy VP, Obrenovich ME, Atwood CS, et al. Involvement of Maillard reactions in Alzheimer disease. *Neurotox Res*. 2002; 4: 191-209.
9) Brownlee M, Vlassara H, Kooney A, et al. Aminoguanidine prevents diabetes-induced arterial wall protein cross-linking. *Science*. 1986; 232(4758): 1629-1632.
10) Nanba F. Effects of black soybean polyphenol on antioxidant and blood flow improvement. Journal of the Brewing Society of Japan. 2013; 108: 413-419. (in Japanese).
11) Ichihashi M, Yagi M, Nomoto K, et al. Glycation stress and photo aging in skin. *Anti-Aging Med*. 2011; 8: 23-29.
12) Tanaka Y, Yagi M, Takabe W, et al. Anti-glycative effect of yogurt: Prevention of advanced glycation end product formation. *Glycative Stress Res*. 2017; 4: 25-31.
13) Hori M, Yagi M, Nomoto K, et al. Experimental models for advanced glycation end product formation using albumin, collagen, elastin, keratin and proteoglycan. *Anti-Aging Med*. 2012; 9: 125-134.
14) Oki T, Osame M, Masuda M, et al. Simple and rapid spectrophotometric method for selecting purple-fleshed sweet potato cultivars with a high radical-scavenging activity. *Breed Sci*. 2003; 53: 101-107.
15) Oki T, Masuda M, Furuta S, et al. Radical scavenging activity of fried chips made from purple-fleshed sweet potato. *Jpn Soc Food Sci Technol*. 2001; 48: 926-932 (in Japanese). doi: 10.3136/NSKKK.48.926$
16) Oki T, Matsui T, Matsumoto K. Evaluation of alpha-glucosidase inhibition by using an immobilized assay system. *Biol Pharm Bull*. 2000; 23: 1084-1087. doi: 10.1248/bpb.23.1084$
17) Bailey T, Bode BW, Christiansen MP, et al. The performance and usability of a factory-calibrated flash glucose monitoring system. *Diabetes Technol Ther*. 2015; 17: 787-794.
18) Yagi M, Shimode A, Yasui K, et al. Effect of a vinegar beverage containing indigestible dextrin and a mixed herbal extract on postprandial blood glucose levels: A single-dose study. *Glycative Stress Res*. 2014; 1: 8-13.
19) Ogura M, Yagi M, Nomoto K, et al. Effect of grapefruit intake on postprandial plasma glucose. *Anti-Aging Med*. 2011; 8: 60-68.
20) Yagi M, Kishimura Y, Okuda F, et al. Effect of yogurt on postprandial blood glucose after steamed riceintake. *Glycative Stress Res*. 2018; 5: 68-74.
21) Matsushima M, Yagi M, Hamada U, et al. Prevention of postprandial hyperglycemia by the combination of a staple food and a side dish. *Glycative Stress Res*. 2014; 1: 53-59.
22) Kawabata A, Yagi M, Ogura M, et al. Postprandial blood glucose level after intake of a bowl of rice topped with beef. *Glycative Stress Res*. 2015; 2: 67-71.
23) Japanese Association of the Study for Glycemic Index. Unified protocol (unified procedure). (in Japanese) http://www.gikenkyukai.com/protocol.html
24) Kwak JH, Kim M, Lee E, et al. Effects of black soy peptide supplementation on blood pressure and oxidative stress: A randomized controlled trial. *Hypertens Res*. 2013; 36: 1060-1066.
25) Tateiwa N, Tamaru S, Iwanaga H, et al. Effects of black soybean intake on blood pressure and lipid metabolites in spontaneously hypertensive rats (SHR). *Abstract Book of the Annual Meeting of the Japanese Society of Nutrition and Food Science*. 2015; ACN2015: 276. (abstract in Japanese)
26) Udagawa H, Hanaoka S, Masuda M, et al. Long-term intake of black soybean extract and extract powder reduces blood pressure in spontaneously hypertensive rats. *Abstract Book of the Annual Meeting of the Japanese Society of Nutrition and Food Science*. 2007; 61: 161. (abstract in Japanese)
27) Kowatari M, Seino N, Karimata T, et al. Effect of black bean extract on fatty acid composition of adipose tissue. *IEICE Technical Report*. 2012; 111: 197-200. (in Japanese) doi:10.1148/gijutsukyo.111.482.197
28) Sato D, Kowatari M, Seino N, et al. Effect of black soybean extract on fatty acid distribution in adipose tissue of high-fat diet-fed rats. *Journal of the Japan Diabetes Society*. 2012; 55(Suppl 1): S-115. (abstract Japanese)
29) Yamashita Y, Okabe M, Natsume M, et al. Cinnamtannin A2, a tetrameric procyanidin, increases GLP-1 and insulin secretion. *Biosci Biotechnol Biochem*. 2013: 77: 888-891.
30) Inaguma T, Han J, Isoda H. Effect of black bean anthocyanins on insulin resistance. *Associate Journal of Japanese Society for Medical Use of Functional Foods*. 2011; 7: 122. (in Japanese)
31) Inaguma T, Han J, Isoda H. Effect of black soybean extract on 3T3-L1 cell differentiation. *Proceeding of the Japanese Biochemical Society and Annual Meeting of the Molecular Biology Society of Japan*. 2010; 83・33: 1P-1102. (abstract in Japanese)
32) Otani J, Ishikawa I, Ishida H, et al. The usefulness of bone-strengthening supplements aimed at improving bone mass. *The Journal of Hiroshima Dental Association*. 2009; 36: 24-30. (in Japanese)

33) Toda T. Evidence-based food development for bone health: "Soybean sprout tea" and "Black soybean tea" as foods for specified health uses for those concerned about bone health. *Clin Cal*. 2006; 16: 1693-1699. (in Japanese) doi: 10.11470/clincalz.16.1693
34) Liu Y, Fukuwatari Y, Kikuchi Y. Research on the effects of black bean broth on functional pathology (Mibyo). *Eastern Medicine*. 2001; 17: 15-27. (in Japanese)
35) Yoshiki T, Ohi N, Matsunaga Y. The effect of black soybean natto intake on menopausal symptoms. *Japanese Journal of Maternal Health*. 2005; 46: 190. (abstract in Japanese)
36) Hirokawa K. Mood-improving effects of black soybean natto: Targeting pre- and postmenopausal women. *Proceedings of The Japanese Psychological Association*. 2007; 71: 1258. (abstract in Japanese)
37) Nakata K, Kaneko A, Sakane Y, et al. Research on functional components and effective use of health tea drinks: (Part 2) Isoflavones and anthocyanins in black soybean tea. *Bulletin of Shizuoka Institute of Environment and Hygiene*. 2007; 49: 41-46. (in Japanese)
38) Kitagawa I, Yoshikawa M, Hayashi T, et al. Characterization of saponin constituents in soybeans of various origins and quantitative analysis by gas -lipid chromatography. *Yakugaku Zasshi*. 1984; 104: 162-168. (in Japanese) doi: 10.1248/yakushi1981.104.2_162
39) Yamagishi T. Herbal medicine series: Black beans (Kuromame). *Medical Kanpo*. 2010; 458: 39-40. (in Japanese)
40) Hashimoto N, Oki T, Sasaki K, et al. Black soybean seed coat extract prevents hydrogen peroxide-mediated cell death via extracellular signal-related kinase signalling in HepG2 cells. *J Nutr Sci Vitaminol (Tokyo)*. 2015; 61: 275-279.
41) Kim JM, Kim KM, Park EH, et al. Anthocyanins from black soybean inhibit Helicobacter pylori-induced inflammation in human gastric epithelial AGS cells. *Microbiol Immunol*. 2013; 57): 366-373.
42) Yamashita R, Kawao N, Nagai N, et al. Effect of black soybean anthocyanins on antithrombotic activity. *Japanese Journal of Pathophysiology*. 2009; 18: 26. (in Japanese)
43) Yamashita R, Kawao N, Ishida C, et al. Antithrombotic effect of black soybean anthocyanins. *Japanese Journal of Thrombosis and Hemostasis*. 2008; 19: 711. (in Japanese)
44) Hoshida S, Miki T, Nakagawa T, et al. Different effects of isoflavones on vascular function in premenopausal and postmenopausal smokers and nonsmokers: NYMPH study. *Heart Vessels*. 2011; 26: 590-595.
45) Okazaki Y, Iqbal M, Kawakami N, et al. A beverage containing fermented black soybean ameliorates ferric nitrilotriacetate-induced renal oxidative damage in rats. *J Clin Biochem Nutr*. 2010; 47: 198-207.
46) Chen YF, Chiang ML, Chou CC, Lo YC. Enhancing the antitumor cell proliferation and Cu2+-chelating effects of black soybeans through fermentation with Aspergillus awamori. *J Biosci Bioeng*. 2013; 115: 400-404.
47) Matsushita I, Kaneyuki T, Koyabu T, et al. Evaluation of the functionality of fermented black soybean extract. *Abstracts of Japan Society of Nutrition and Food Science*. 2007; 61: 157. (abstract in Japanese)
48) Shon YH, Lee HS, Kim CH, et al. Effect of herbal medicines on cytokine-induced cytotoxicity and major histocompatibility complex class II antigen expression in rat thyroid cells. *Biol Pharm Bull*. 2004; 27: 371-374.
49) OhZuiun (Wang R). Mother's Warm Hand Healing: Beans (Part 2) Black Beans. *Kampo Kenkyu (Journal of Kampo Research)*. 2004; 387: 108-111. (in Japanese)
50) Mikami N, Fujino K, Shigeta M, et al. Evaluation of antioxidant and α-glucosidase inhibitory activities of blended health tea and its raw tea leaves. *Journal of the University of Yamaguchi Prefectural University*. 2013; 6: 5-11. (in Japanese)
51) Yoshida M, Hosaka S, Kazuoka T, et al. Health effects of black soybean citric acid vinegar in growing rats. *The Japanese Journal of Health and Medical Science*. 2006; 15: 48-49. (abstract in Japanese)
52) Hosaka K, Sooka T, Tsutsumi K, et al. Development of a healthy beverage containing citric acid using black soybeans. *Proceeding of Annual General Meeting of the Japanese Society of Health and Medical Science*. 2006; 15: 36-37. (abstract in Japanese) doi: 10.20685/kenkouigaku.15.3_36
53) Hosaka K, Sooka T, Tsutsumi K, et al. Development of a healthy beverage containing citric acid using black soybeans. *Journal of Japan Health Medicine Association*. 2008; 17: 19-24. (in Japanese) doi: 10.20685/kenkouigaku.17.2_19
54) Ochiai H, Taniguchi T, Kodama T, et al. Evaluation of health promoting effects of black soybean extract containing citric acid beverage in humans. *Abstracts of the General Meeting of the Japanese Society of Public Health*. 2004; 63: 864. (abstract in Japanese)
55) Kato Y, Hayashibara K, Zhu J. Preparation of black soybean lactic acid fermented food with improved functionality: Evaluation of antioxidant capacity and constipation-improving effect in mice. *Abstract Book of the Annual Meeting of the Japanese Society of Nutrition and Food Science*. 2007; 61: 171. (abstract in Japanese)
56) Hashimoto Y. Recommended recipes for osteoporosis prevention! Summer rice with ume, black beans, and small fish. *Nutrition Care*. 2018; 11: 739. (abstract in Japanese)
57) Nio K, Aso M. My Snack friendly to blood sugar level: (Part5) November snack (Cottage cheesecake/Black bean agar). *Diabetes Care*. 2017; 14: 986-988. (in Japanese)
58) Yamai M, Tsumura K, Kimura M, et al. Antiviral activity of a hot water extract of black soybean against a human respiratory illness virus. *Biosci Biotechnol Biochem*. 2003; 67: 1071-1079.
59) Kawamura F. Eating iron (1): Boiling black beans in an iron pot. *The Japanese Journal of Clinical Nutrition*. 1982; 60: 115. (abstract in Japanese)
60) Hagiwara A, Imai N, Numano T, et al. A twenty eight-day repeated dose toxicity study of black soybean extract in Sprague-Dawley rats. *J Toxicol Sci*. 2010; 35: 87-96. doi: 10.2131/jts.35.87
61) Hagiwara A, Imai N, Numano T, et al. 28-day repeated dose toxicity study of black soybean extract in rats. *J Toxicol Sci*. 2009; 34(Suppl): S193. (abstract in Japanese)
62) Yoshizaki F, Houga M, Takada M, et al. Effects of herbal drugs on the acute toxicity of Aconiti tuber in mice. *Journal of Traditional Medicines*. 1993; 10: 161-165. (in Japanese)

63) Adachi A, Hamaoka D, Moriyama T. Three cases of anaphylaxis due to soy protein-containing health drinks, health foods, and black bean tea. *Japanese Journal of Clinical Dermatology*. 2022; 64: 1476-1481. (in Japanese)
64) Arata K, Mineoka R, Ioka N, et al. A case of food-dependent exercise-induced anaphylaxis due to yuba (soy milk skin). *Japanese Journal of Clinical Dermatology*. 2020; 62: 1559-1563. (in Japanese)
65) Ario K, Mizuta Y, Nagatsuma G, et al. A case of iron overload possibly caused by black soybean intake. *Program and Abstract Book of the Kyushu Regional Meeting of the Japanese Society of Gastroenterology and the Kyushu Regional Meeting of the Japan Gastroenterological Endoscopy Society*. 2022; 119・113: 166. (abstract in Japanese)
66) Tamura T, Matsushima T, Miyakawa A, et al. A case of esophageal burn caused by black bean soup. *Gastroenterological Endoscopy*. 2011; 53(Suppl 1): 834. (in Japanese)
67) Yagi M, Yonei Y. Glycative stress and anti-aging: 2. The Evaluation of Glycative Stress: Measurement of blood glucose, glycated proteins and intermediates. *Glycative Stress Res*. 2016; 3: 205-209.
68) Ishioka Y, Yagi Y,Ogura M, Yonei Y. Antiglycation effect of various vegetables: Inhibition of advanced glycation end product formation in glucose and human serum albumin reaction system. *Glycative Stress Res*. 2015; 2: 22-34.