The Functional Components and Functionalities of Scarlet Runner Beans

（花豆の機能性成分と機能性に関する研究）

Bioresources Science

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I. Introduction

An imbalance between energy intake and expenditure may result in the abnormal growth of adipose tissue, thereby leading to obesity [1]. Obesity is strongly associated with the metabolic syndrome, which is a risk factor for several lifestyle diseases, such as hyperlipidemia, hypertension, and diabetes [2]. Diabetes has received considerable attention owing to the increasing number of people suffering from this chronic disease [3]. Postprandial hyperglycemia is an important risk factor for the development of Type II diabetes [4]. Controlling the blood glucose level is the most effective method for preventing diabetes deterioration and hyperglycemia [5]. All dietary carbohydrates are hydrolyzed by enzymes to yield simple sugars, which can improve blood glucose levels [6].

According to the World Health Organization (WHO), limiting energy intake from total fats and sugars could suppress overweight and obesity as well as their related diseases, engaging in regular physical activity, and increasing consumption of fruits, vegetables, legumes, whole grains, and nuts. Dietary intervention seems to be an effective option for treatment of obesity and type 2 diabetes. Inhibition of dietary fat and sugar absorption from the intestine seems to be an effective way to prevent obesity and type 2 diabetes.

α-Glucosidase is a membrane-bound enzyme located in the epithelium of the small intestine, and it catalyzes the cleavage of glycosidic bonds and releases of glucose from disaccharides and oligosaccharides [7]. Pancreatic α-amylase is an endoglucosidase that is delivered into the intestinal lumen as a constituent of pancreatic juices and catalyzes the hydrolysis of starch to maltose and maltotriose [7]. Pancreatic lipase is a key enzyme for fat digestion, blocking fat decomposition and absorption by its inhibition is an effective approach for preventing obesity. Some researchers have reported that many kinds of plants had potential α-glucosidase, α-amylase, and lipase inhibitory activities [8,9,10,11,12].

Proanthocyanidins belong to flavonoids and are oligomers or polymers of flavan-3-ols [13].

They can be classified into two subgroups, namely B-type proanthocyanidins, in which monomers are linked only with B-type bonds, and A-type proanthocyanidins, which have both A- and B-type bonds. The mean degree of polymerization (mDP) is a factor influencing the biological activities of proanthocyanidins. For example, polyphenolic compounds with higher mDP have been shown to possess greater antioxidant [14] and lipase inhibition activities [15]. Thiolysis is a method for the structural characterization of proanthocyanidins, as it supposedly only breaks the single B-type bonds without affecting the doubly linked A-type bonds, resulting in the release of the terminal units and the formation of thioether derivatives from the extension units. For example, thiolysis in okra results in the release of underivatized epigallocatechin and epicatechin [16], whereas epigallocatechin and gallocatechin are released as derivatives by the cleavage of B-type proanthocyanidins bonds of that takes place during thiolysis in pea and faba bean [17].

Legumes are important food sources for humans in many developing countries; in addition to protein, carbohydrates (dietary fiber), minerals, and vitamins, they contain a wide range of phytochemicals, including phenolics with antioxidant and other bioactivities. Anti-inflammatory activities of phenolic compounds have been detected in white and red common beans [18] and *Phaseolus angularis* beans [19]. Scarlet runner bean (*Phaseolus coccineus* L.) is cultivated for its seeds (dried or fresh), but is also an ornamental plant [20]. The dry seeds are used in salads, soups, and amanatto in Japan. The seed coat of legume grains was reported to contain numerous types of phenolics, which are suggested to play an important protective role against oxidative damage in consumer's body [21]. The distribution of phenolic compounds differs in the cotyledon and the seed coat, with non-flavonoid phenolic compounds, such as free and combined hydroxybenzoic and hydroxycinnamic acids, being located mainly in the cotyledon of lentils [22]. Flavonoids, such as glycosides of flavonols and flavones, were identified in the seed coat of lentils, together with trans-resveratrol-3-O-

glucoside, and higher concentrations of proanthocyanidins [22].

Legume must be processed prior to consumption (such as germinating, roasting, soaking and cooking) in order to improve their palatability and nutritional quality, and these processes produce changes in their nutritional composition and content of bioactive compounds [23] [24]. Cooking brings about a number of changes in the physical characteristics and chemical composition of food legumes. Food legumes are usually cooked by a boiling process before use. Pressure boiling and steaming can also be used for this purpose. High pressure processing technology may provide high quality (flavour, colour and biological active components) food products [25]. The phenolic components and chemical antioxidant activities of thermal processed common beans and soybeans have been investigated by Xu & Chang, 2008 [26]. Nuria E. Rocha-Guzma´n, 2007 [27] reported that great influence of cooking pressure time in phenols distribution in seed coat, cotyledon and cooking water of common beans. These preliminary studies showed that soaking, boiling, and steaming processing significantly affected the phenolic components and antioxidant activities (determined by in vitro chemical assays) of the beans studied.

In the present study, thiolysis was utilized to structurally characterize the polyphenols contained in the purple scarlet runner bean's coat. Their ability to inhibit digestive enzymes, including α-glucosidase, pancreatic α-amylase and pancreatic lipase in vitro, and the effect of polyphenols from purple scarlet runner bean's coat on blood glucose levels after the oral administration of starch in mice and their anti-obesity effect in high-fat diet-fed mice in vivo were examined. Moreover, the effect of boiling pressure on the polyphenol compounds and the functionality in purple scarlet runner beans were investigated.

II. Antioxidant and α-Glucosidase Inhibitory Activity of Various Scarlet Runner Bean Polyphenols

1. Materials and Methods

1.1. Materials

5 types of scarlet runner beans (SRB) were purchased from the Kawanishi Agricultural Cooperative Association (Obihiro, Japan). The colors of the seed coat from SRB (black), SRB (brown), and SRB (white) were pure. SRB (purple) had black spots on a purple surface, and SRB (mixed) had many black or brown spots on a cream surface (Figure 1). The weight of 100 grains of SRB ranged from175.2 g to 220.7 g. Diaion HP-20 columns and Sephadex LH-20 columns for chromatography were obtained from the Mitsubishi Chemical Corporation (Tokyo, Japan) and GE Healthcare Bio-Sciences AB (Uppsala, Sweden), respectively. All other reagents and chemicals were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan), unless stated otherwise.

1.2. Color Measurement

Seed coat color was determined using a Minolta CR-200 Chroma Meter (Minolta, Tokyo, Japan). L*, a* and b* values were determined. The L* value represents lightness, a* represents greenness and redness, and b* represents blueness and yellowness. A white porcelain plate (L* = 97.75, a* = −0.08, and b* = +1.77) supplied with the instrument was used for calibration. The following formula was used to calculate the C (chroma) value:

$$C=\sqrt{a^{*2}+b^{*2}} \quad (1)$$

1.3. Extract Preparation and Fractionation

SRB were ground into a powder, followed by extraction with 20 mL of 80% ethanol. After ultrasound treatment for 30 min, the mixture was centrifuged at 1,006 × *g* for 10 min to obtain a supernatant. The same extraction process was repeated two more times. The residues were subjected to another three rounds of extraction with 70% acetone and the supernatant. Then, the supernatant was mixed, concentrated by rotary evaporation in a vacuum, and purified by chromatography through Diaion HP-20 columns. The columns were washed with distilled water and then eluted with methanol. The methanol solution was concentrated by rotary evaporation in a vacuum and dissolved in 2 mL of methanol for the experiment. Part of the concentrate was dissolved in ethanol and fractionated by Sephadex LH-20 column chromatography. The column was successively eluted with ethanol, methanol, and 60% acetone to collect fraction I (Fra.I), fraction II (Fra.II), and fraction III (Fra.III), respectively.

1.4. Quantification of Polyphenols

Polyphenols were quantified using the Folin–Ciocalteu method [28]. The methanol fraction (HP-20 column) (100 μL) was treated with 300 μL of distilled water, 400 μL of Folin–Ciocalteu reagent, and 400 μL of a 10% Na2CO3 solution. The mixture was prepared in triplicate, incubated at 30°C for 30 min, and centrifuged at 1,006 × *g* for 10 min. The absorbance of the mixed supernatant was measured at 760 nm. The polyphenol content is expressed in mg of catechin equivalents per gram of beans (mg/g).

1.5. Quantification of Procyanidins

The procyanidin content of the methanol fraction (HP-20 column) was determined by the HCl-butanol method [29] using cyanidin as the standard equivalent.

1.6. Estimation of DPPH Radical Scavenging Activity

DPPH radical scavenging activity was evaluated by the method described by Brand-Williams et al. [30], with some modifications. A 50-μL aliquot of the methanol fraction (HP-20 column) was mixed with 100 μL of ethanol, and the mixture was supplemented with 150 μL of 0.5 mM DPPH in ethanol. The absorbance of the mixture was measured using a microplate reader at 517 nm. The DPPH radical scavenging activity is expressed in μmol trolox equivalents per gram of beans (μmol/g).

1.7. Estimation of Reducing Power

Reducing power was evaluated according to a previously reported method, with minor modifications [31]. Briefly, 250 μL of the methanol fraction (HP-20 column) was mixed with 250 μL of sodium phosphate buffer (pH 7.5) in a test tube, and 250 μL of 1% (w/v) potassium ferricyanide was added. The mixture was incubated at 50°C for 20 min. After the incubation period, 250 μL of 10% trichloroacetic acid was added and centrifuged at 1,006 × *g* for 10 min. The upper layer of the supernatant (500 μL) was mixed with 500 μL of distilled water and 100 μL of 0.1% (w/v) ferric chloride, and reacted under shade for 15 min. The absorbance of the reaction mixture was measured at 700 nm. Reducing power activity is expressed in mg of vitamin C equivalent per gram of beans (mg/g).

1.8. α-Glucosidase Inhibitory Activity

α-Glucosidase inhibition was analyzed following the methods of Matsumoto et al. [32], with modifications. Sucrose was broken down by α-glucosidase, and the amount of reducing sugar was calculated based on the α-glucose content. In total, 0.8 mL of the enzyme reaction solution (50 μL of 0.4% sucrose, 625 μL of 0.1 mol/L sodium phosphate buffer (pH 6.8), and 125 μL of 1% NaCl) was pre-incubated at 37°C for 30 min. The methanol fraction (HP-20 column) was concentrated by rotary evaporation in a vacuum and dissolved in distilled water.

An aqueous solution (polyphenol concentration, 0–100 μg/mL) was added to 0.1 U/mL α-glucosidase (EC3.2.1.20; Oriental Yeast Co., Ltd., Tokyo, Japan) at 37°C for 10 min. After pre-incubation, 200 μL of the mixture (polyphenol extract and α-glucosidase) was added to the enzyme reaction solution and incubated at 37°C for 30 min. The reaction was terminated by adding 125 mL of 2 M NaOH, and 1% dinitrosalicylic acid was added in boiling water for 10 min. After incubation, the mixture was analyzed at 540 nm at room temperature. Enzyme inhibitory reactions for all polyphenol extract concentrations were replicated three times. The α-glucosidase inhibitory activity is expressed as the percent inhibition. The concentration of inhibitors required for the inhibition of 50% of the enzyme activity under the assay conditions was defined as the IC50 value.

1.9. Statistical Analysis

Values are presented as means ± standard error. Statistical significance was evaluated by ANOVA and least significant difference (LSD) tests (SAS Enterprise Guide 5.1). Differences were considered significant when $p < 0.05$.

2. Results and Discussion

2.1. Color Variation

Consumers initially evaluate foods by their surface color; accordingly, this quality parameter is critical for the acceptance of the product. We used a chroma meter to determine the colors of SRB. Table 1 summarizes the L*, a*, b*, and C values. SRB (mixed) had too many spots on a cream surface; therefore, the values are not shown. SRB (white) showed the highest L* value (70.7), and SRB (black) and SRB (brown) showed the lowest values ($p < 0.05$). These results were consistent with those of Ci et al. [33], who showed that SRB (white) has the highest L* value in an analysis of 30 kinds of seeds. The a* and b* values were -0.4–

2.1 and 0.2–8.8, respectively. Faba beans had a* and b* values of 5 and 24, respectively [34]. The C value was highest for SRB (white) (8.8) and lowest for SRB (black) (0.4).

2.2. Polyphenol and Procyanidin Content, DPPH Radical Scavenging Activity, and Reducing Power

Table 2 summarizes the polyphenol and procyanidin contents, DPPH radical scavenging activity, and reducing power. SRB (purple) and SRB (brown) showed the highest polyphenol contents of 9.9 and 9.7 mg/g ($p < 0.05$). These values were greater than those of *Vigna angularis* (willd.) Ohwi et H. Ohashi and *Phaseolus vulgaris* L. [33]. We found a positive correlation ($r = 0.89$) between the polyphenol content and a* value for SRB (except SRB (mixed)). The same results have been obtained using the seed coats of red beans [33]. These results could provide useful information for customers choosing beans with a higher polyphenol content by the surface color. The procyanidin contents were 4.6, 3.9, and 3.8 mg/g for SRB (brown), SRB (purple), and SRB (black), and these values were higher than those of SRB (white) and SRB (mixed). SRB (brown) and SRB (mixed) showed the highest DPPH radical scavenging activity and reducing power. SRB (white) showed the lowest polyphenol and procyanidin contents, DPPH radical scavenging activity, and reducing power. A positive correlation was found between polyphenol content and DPPH radical scavenging activity (correlation coefficient, 0.98), and between polyphenol content and reducing power (correlation coefficient, 0.88) (Figure 2). Many researchers have previously found a positive correlation between the polyphenol content and DPPH radical scavenging activity for the pods of common beans [35], legumes in India [36], and thirteen genotypes of faba beans [37]. Moreover, polyphenols in seed coat and cotyledon from SRB (purple), SRB (black) and SRB (white) showed in Figure 3. Moreover, the proportion of seed coat polyphenols were 84.23% in 1 g of scarlet runner beans (purple), and those were 72.75% in scarlet runner beans (black).

The seed coat exhibited higher polyphenol content in scarlet runner beans (black) and scarlet runner beans (purple). However, the proportion of seed coat polyphenols were 20.89%, and those of cotyledon were 79.11% in 1 g of scarlet runner beans (white).

2.3. Polyphenol Fractions

We performed Sephadex LH-20 column chromatography to obtain three polyphenol fractions, i.e., Fra.I, Fra.II, and Fra.III, for each SRB (Figure 4). According to Saito et al. [38], Fra.I contains monomeric polyphenols, Fra.II contains oligomeric polyphenols, and Fra.III contains polymeric polyphenols. SRB (white) had mostly Fra.I (79.7%) with small amounts of Fra.II (20.3%), and Fra.III was not detected. In contrast, the other SRB showed a higher ratio of Fra.II (>63.9%) (Table 3). SRB (white) showed a lower ratio of oligomeric and polymeric polyphenols and a lower DPPH radical scavenging activity and reducing power than those of other SRB. We found that oligomeric polyphenols possess greater DPPH radical scavenging activity than that of monomeric polyphenols [35]. Moreover, we analyzed Fra.II and Fra.III by MALDI-TOF/MS. SRB (white) contained catechin and gallocatechin as basic units constituting oligomeric polyphenols; however, the other SRB contained catechin as a basic unit constituting oligomeric or polymeric polyphenols. The basic unit structure may also be a factor determining the antioxidant activity. In a previous study, Lu et al. [16] detected gallocatechin as a basic unit in polymeric polyphenols of okra seeds by MALDI-TOF/MS.

2.4. α-Glucosidase Inhibitory Activity

We analyzed the inhibitory effects of polyphenols on α-glucosidase. Polyphenols from SRB inhibited the activity of α-glucosidase in a dose-dependent manner. On α-glucosidase, the polyphenols (50 μg/mL) for SRB (black) showed the highest inhibitory activity (85.7%), and those for SRB (white) showed lowest inhibitory activity (53.8%) (Table 4). SRB (black)

showed a greater oligomeric and polymeric polyphenol content (87%) than that for SRB (white) (20.3%). We speculate that oligomeric and polymeric polyphenols had stronger α-glucosidase inhibitory activity than the monomeric polyphenols for SRB. Moreover, SRB (black) had a lower IC_{50} value (26.4 μg/mL), and SRB (white) had a higher IC_{50} value (58.4 μg/mL) those of other SRB. Phenolic compounds from seven legumes [38] and soybeans [39] inhibit α-glucosidase activity. We have previously found that the coat of SRB (purple) can effectively reduce blood glucose after the oral administration of starch in mice [40]. Based on the α-glucosidase inhibitory activity of other SRB in vitro, we speculate that they also had the potential to reduce blood glucose.

We also performed an enzyme kinetic study of α-glucosidase inhibition. Figure 5 shows a Lineweaver–Burk plot of the α-glucosidase inhibitory activity of polyphenols from SRB (black) at 0 and 10 μg/mL, with different concentrations of sucrose (3.3–20.0 mM). The maximum velocity (*V*max) was 0.59 mmol/min and the Michaelis–Menten constant (*K*m) was 20.6 mM for sucrose. When the concentration of polyphenols was 10 μg/mL, *V*max increased to 0.7 mmol/min, and *K*m increased to 31.8 mM. In the presence of polyphenols from SRB (black), both *V*max and *K*m increased, implying that polyphenols exhibited a mixed type of inhibition towards α-glucosidase, i.e., non-competitive and uncompetitive inhibition. It has been reported that the structural factors of phenolic groups are crucial determinants of the inhibitory pattern of polyphenols on α-glucosidase [13]. In our study, both SRB (purple) and SRB (black) had higher levels of polymeric polyphenols than monomeric polyphenols. We speculate that polyphenols from SRB (purple) also have a mixed pattern of α-glucosidase inhibition. Different natural compounds have different inhibition patterns against α-glucosidase; for example, finger millet (*Eleusine coracana L.*) seed coat phenolics exhibit noncompetitive inhibition [41], and three flavonoids (quercetin, isoquercetin, and rutin) exhibit mixed noncompetitive and anticompetitive inhibition [42].

SRB (purple) SRB (brown) SRB (mixed) SRB (black) SRB (white)

Figure 1. The various scarlet runner beans (SRB).

Table 1. Summary of L^*, a^*, b^*, and C values for scarlet runner beans (SRB).

	L*	a*	b*	C
SRB (black)	$31.9^{c} \pm 1.5$	$0.3^{b} \pm 0.2$	$0.2^{c} \pm 0.1$	$0.4^{c} \pm 0.2$
SRB (brown)	$34.0^{c} \pm 1.9$	$2.1^{a} \pm 0.8$	$1.9^{b} \pm 0.8$	$2.8^{b} \pm 1.1$
SRB (purple)	$35.4^{b} \pm 2.1$	$1.9^{a} \pm 1.0$	$1.6^{b} \pm 0.8$	$2.5^{b} \pm 1.2$
SRB (white)	$70.7^{a} \pm 3.9$	$-0.4^{c} \pm 0.1$	$8.8^{a} \pm 1.2$	$8.8^{a} \pm 0.2$

Values followed by different letters within a column are significantly different ($p < 0.05$).

Table 2. Summary of polyphenols, procyanidins, DPPH radical scavenging activity, and reducing power for scarlet runner beans.

	Polyphenols (mg/g)	Procyanins (mg/g)	DPPH radical scavenging activity (μmol/g)	Reducing power (mg/g)
SRB (black)	$7.6^{c} \pm 0.2$	$3.8^{b} \pm 0.1$	$39.3^{c} \pm 0.3$	$5.1^{b} \pm 0.1$
SRB (brown)	$9.7^{a} \pm 0.3$	$4.6^{a} \pm 0.1$	$47.5^{a} \pm 0.2$	$6.5^{a} \pm 0.2$
SRB (purple)	$9.9^{a} \pm 0.1$	$3.9^{b} \pm 0.1$	$41.7^{b} \pm 0.7$	$4.1^{c} \pm 0.2$
SRB (white)	$0.5^{d} \pm 0.1$	$0.4^{d} \pm 0.1$	$0.3^{d} \pm 0.1$	$0.4^{d} \pm 0.0$
SRB (mixed)	$9.1^{b} \pm 0.1$	$3.4^{c} \pm 0.1$	$47.6^{a} \pm 0.5$	$6.7^{a} \pm 0.3$

Values followed by different letters within a column are significantly different ($p < 0.05$).

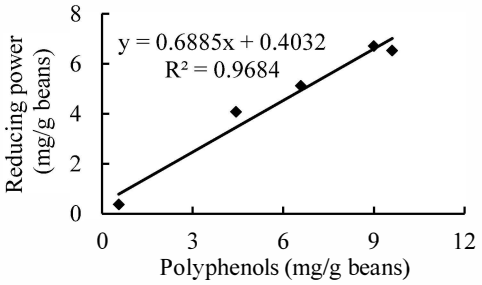

Figure 2. The relationship between polyphenol content and DPPH radical scavenging activity, and between polyphenol content and reducing power in scarlet runner beans (SRB).

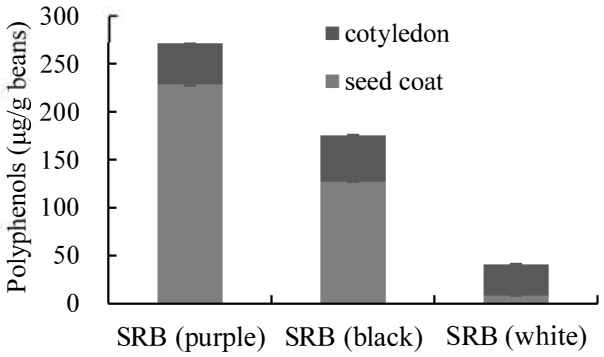

Figure 3. Polyphenols in seed coat and cotyledon from scarlet runner beans (SRB).

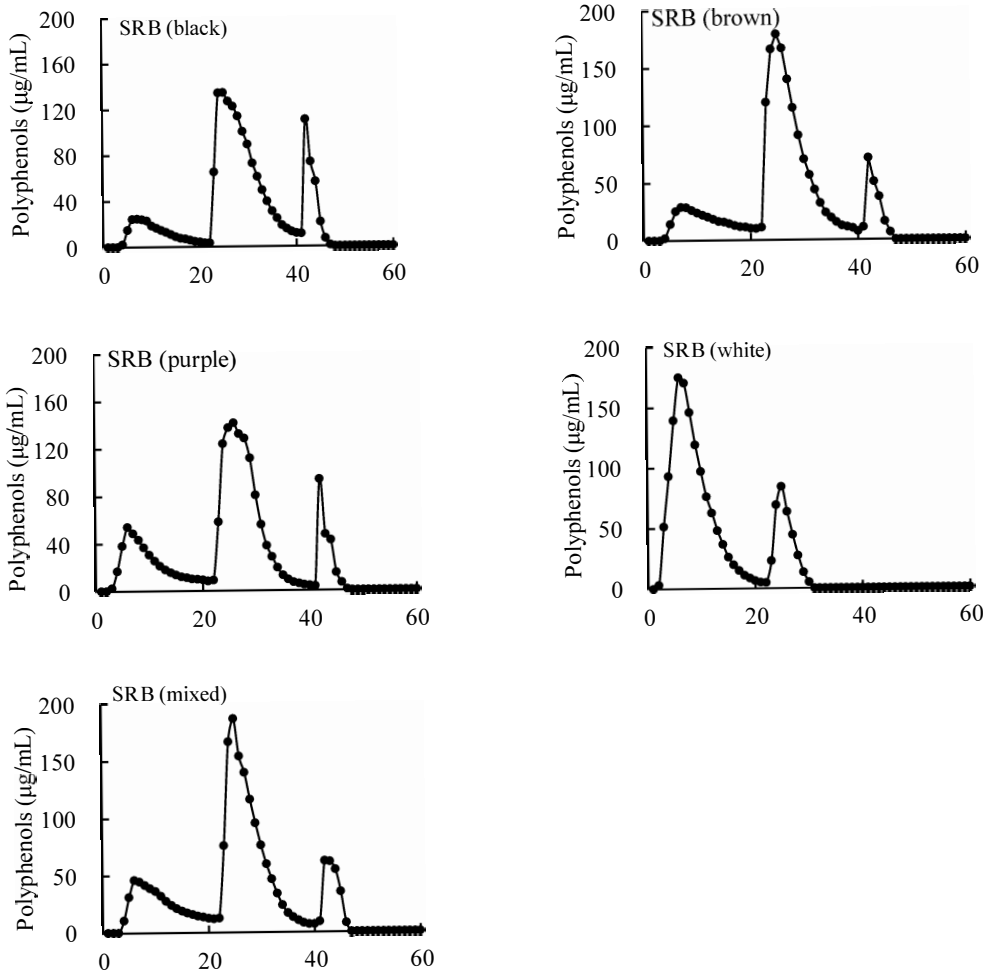

Figure 4. Scarlet runner bean (SRB) polyphenols were applied to the LH-20 column for the successive elution of Fra.I (ethanol fraction, 1–20), Fra.II (methanol fraction, 21–40), and Fra.III (60% acetone fraction, 41–60).

Table 3. Scarlet runner bean (SRB) polyphenols were applied to the LH-20 column for the successive elution of Fra.I (ethanol fraction, 1–20), Fra.II (methanol fraction, 21–40), and Fra.III (60% acetone fraction, 41–60).

	Fra.I (%)	Fra.II (%)	Fra.III (%)
SRB (black)	13.1	71.4	15.6
SRB (brown)	17.4	71.9	10.7
SRB (purple)	24.3	63.9	11.8
SRB (white)	79.7	20.3	N.D.
SRB (mixed)	23.7	64.4	11.9

N.D.: not detected

Table 4. α-Glucosidase inhibitory activity for various scarlet runner beans (SRB).

	50 μg/mL polyphenols (%)	IC_{50} (μg/mL)
SRB (black)	$85.7^{a} \pm 0.1$	26.4
SRB (brown)	$65.8^{c} \pm 0.2$	41.9
SRB (purple)	$69.5^{b} \pm 0.4$	39.7
SRB (white)	$53.8^{e} \pm 0.7$	58.4
SRB (mixed)	$54.7^{d} \pm 0.3$	56.5

Values followed by different letters within a column are significantly different ($p < 0.05$).

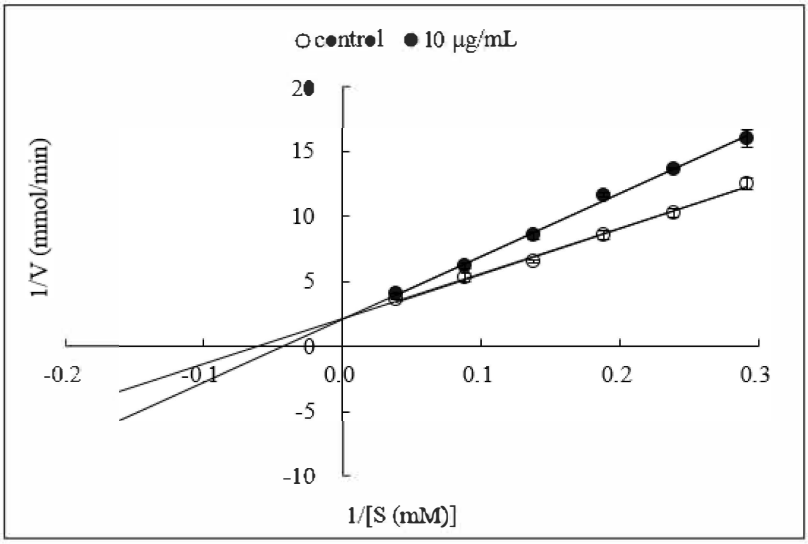

Figure 5. Lineweaver–Burk plots of polyphenols against α-glucosidase for SRB (black).

III. Anti-Obesity on High-Fat Diet-Fed Mice and Suppressive on Blood Glucose Levels of Polyphenols from the Seed Coat of Scarlet Runner Beans (Purple)

1. Introduction

The section. II (Antioxidant and α-Glucosidase Inhibitory Activity of Various Scarlet Runner Bean Polyphenols) analyzed polyphenol and procyanidin content, DPPH radical scavenging activity, reducing power, and α-glucosidase inhibitory activity in five scarlet runner beans, including SRB (purple), SRB (brown), SRB (mixed), SRB (black) and SRB (white). Scarlet runner beans except SRB (white) showed high polyphenol and procyanidin content, DPPH radical scavenging activity, reducing power and α-glucosidase inhibitory activity. SRB (purple) showed the highest polyphenol contents among five types of scarlet runner beans. Moreover, polyphenols of seed coat from SRB (purple) exhibited high proportion than that of cotyledon, the proportion was 84.23%. So thus, in Sub. III, anti-obesity on high-fat diet-fed mice and suppressive on blood glucose levels of polyphenols *in vitro* and *vivo* from the seed coat of scarlet runner beans (purple) were analyzed.

2. Materials and Methods

2.1. Materials

Scarlet runner beans (purple) were obtained from the Kawanishi Agricultural Cooperative Association (Obihiro, Japan). Diaion HP-20 columns and Sephadex LH-20 columns for chromatography were supplied by the Mitsubishi Chemical Corporation (Tokyo, Japan) and GE Healthcare Bio-Sciences AB (Uppsala, Sweden), respectively. All other reagents and chemicals were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan) unless

stated otherwise.

2.2. Extraction and Fractionation of Polyphenols

Scarlet runner beans (purple) were immersed in distilled water and the coat was manually separated from the cotyledon, freeze-dried, grounded into powder, and extracted with 70% acetone. The mixture was filtered using Advantec No. 5 filters (Tokyo, Japan) and the filtrate was collected. The residues were subjected to another four rounds of 70% acetone extraction and filtration. Filtrates and soaking water were mixed, concentrated by rotary evaporation under vacuum, and purified by chromatography through Diaion HP-20 columns. The columns were washed with distilled water and then eluted with methanol. The SRPA-containing methanol fraction was concentrated by rotary evaporation under vacuum and used to supplement the mouse diet in the animal experiments. Moreover, a part of the concentrate was dissolved in ethanol and fractionated by Sephadex LH-20 column chromatography. The column was successively eluted with ethanol, methanol, and 60% acetone, to collect fractions I (fra.I), fraction II (fra.II), and fraction III (fra.III), respectively. The polyphenol content of was determined by Folin–Ciocalteu method [28]. The proanthocyanidin content was determined by the HCl-butanol method [29], using cyanidin as the standard equivalent. DPPH radical scavenging activity was evaluated by the method described by Brand-Williams et al. [30], with some modifications.

2.3. Thiolysis of SRPAs for RP-HPLC Analysis

Thiolysis was conducted according to a reported method with minor modification [43]. In a small glass vial, fra.II or fra.III (250 mg/mL) was mixed with HCl (0.1 % v/v in methanol) and 2-mercaptoethanol (5% v/v in methanol). The vial was sealed with an inert Teflon cap, heated at 40 ℃ for 60 min, and then stored at −20 ℃ until analysis. RP-HPLC (reversed-

phase high-performance liquid chromatography) separation was conducted on C18 columns (250 mm × 4.6 mm) (Shimadzu Corporation, Tokyo, Japan), with SPD-10AD (Shimadzu Corporation, Tokyo, Japan) used for detection. Analysis was performed with (A) 0.1% (v/v) trifluoroacetic acid and (B) 0.1% (v/v) trifluoroacetic acid-acetonitrile. The elution protocol was as follows: 8% B (initial), 30% B for 30 min, 30% B for 50 min, and 8% B for 60 min at a flow rate of 1 mL/min. Detection was performed at 280 nm. The mDP of proanthocyanidins was calculated using the following formula:

$$mDP = 1 + \frac{area\ of\ [\text{catechin and epicatechin derivatives}]}{area\ of\ [\text{catechin and epicatechin}]} \quad (2)$$

2.4. Determination of Pancreatic Lipase Activity in Vitro

The assay for determining pancreatic lipase activity in vitro was carried out with a slightly modified version of the protocol described in Han et al. [44]. Briefly, 30 μL of 3 mg/mL pancreatic lipase and 0.45 mL of various concentrations of sample solution were pre-incubated for 10 min at 37 °C. After pre-incubation, a substrate solution containing glycerol triolein (80 mg), phosphatidylcholine (10 mg) and cholic acid (5 mg) in 9 mL of 0.1 M N-[tris(hydroxymethyl)methyl]-2-aminoethanesulfonic acid buffer (pH 7.0) was added and incubated for 30 min at 37°C. Afterward, 2.5 mL of 1 M copper reagent and 5 mL chloroform were added to the reaction mixture, which was then centrifuged at 1,006 ×*g* for 10 min. The upper, aqueous phase was removed, whereas sodium diethyldithiocarbamate was added to the lower, free fatty acid-containing chloroform phase. The absorbance was measured at 440 nm and lipid concentrations were calculated using linoleic acid as a standard equivalent. Lipase inhibitory activity (%) was calculated from the following formula:

$$\text{Lipase inhib. activity}(\%) = \left(1 - \frac{FFA_{sample}}{FFA_{control}}\right) \times 100\% \quad (3)$$

where *FFAsample* and *FFAcontrol* are the quantities of free lipids in the sample and the

control, respectively.

2.5. α-Glucosidase Inhibitory Activity and α-Amylase Inhibitory Activity

α-Glucosidase and α-amylase inhibitory activity were analyzed following the methods of Matsumoto et al. [32] with modifications. In total, 0.8 mL of enzyme reaction solution [50 μL of 0.4% sucrose for α-glucosidase inhibitory activity and 0.5% starch for α-amylase inhibitory activity, 625 μL of 0.1 mol/L sodium phosphate buffer (pH 6.8), and 125 μL of 1% NaCl] was pre-incubated at 37 ℃ for 30 min. The methanol fraction (after HP-20 column) was concentrated by rotary evaporation in a vacuum at 35 ℃ and dissolved in distilled water. Distilled water fraction was added to 0.1 U/mL yeast α-glucosidase (EC3.2.1.20; Oriental Yeast Co., Ltd., Tokyo, Japan) solution and porcine pancreatic α-amylase (EC3.2.1.1; Sigma-aldrich Inc. Co., LLC, Steinheim, Germany) solution at 37 °C for 10 min. After pre-incubation, 200 μL of the mixture (polyphenol extract and α-glucosidase, α-amylase) was added to the enzyme reaction solution and incubated at 37 °C for 30 min. The reaction was terminated by adding 125 mL of 2 M NaOH and 1% dinitrosalicylic acid in boiling water for 10 min. After incubation, the mixture was analyzed at 540 nm at 25 °C. Enzyme inhibitory reactions for all polyphenol extract concentrations were replicated three times. The enzyme inhibitory activity is expressed as the percent inhibition. The concentration of inhibitors required for the inhibition of 50% of the enzyme activity under the assay conditions was defined as the IC_{50} value.

2.6. Animals and Diets

Male ddy mice (Japan SLC, Inc., Shizuoka, Japan) were housed in plastic cages at 23 °C on a 12 h/12 h light/dark cycle, at a relative humidity of 60%. Mice had free access to standard chow and water for one week before the experiment. During the experiment, mice were fed

a high-fat diet (Quick Fat; CLEA Japan, Inc., Tokyo, Japan) or standard chow (CE-2; CLEA Japan) to serve as controls. The high-fat diet had a total calorie content of 415.1 kcal/100 g and its composition (w/w %) was as follows: 24.0% crude protein, 14.6% crude fat, 2.7% crude fiber, 5.1% crude ash, 46.7% NFE (nitrogen-free extract), and 7.3% moisture [45]. Kishida et al. [41] used the same high-fat diet to induce obesity and type-2 diabetes in their study on the use of brown adipocytes to ameliorate insulin resistance. Standard chow had a total calorie content of 344.9 kcal/100 g and contained (w/w %) of 24.9% crude protein, 4.6% crude fat, 4.1% crude fiber, 6.6% crude ash, 51.0% NFE, and 8.9% moisture [41]. Oral Starch Tolerance Test in Mice was used the standard chow. The study was approved by the regulatory authority of the National University Corporation Obihiro University of Agriculture and Veterinary Medicine, and it adhered to the standard principles described in the Guide for the Care and Use of Laboratory Animals.

2.7. Anti-obesity Evaluation in Mice Fed a High-Fat Diet

After adaptation, mice were divided into four groups, each with seven subjects: (1) high-fat diet group (HF); (2) high-fat diet with 0.5% SRPA (0.5% SRPAHF) group; (3) high-fat diet with 1.0% SRPA (1.0% SRPAHF) group; (4) standard chow (SC) group. During the experiment, food and water were provided *ad libitum*. Food and water intake, body weight, and the weight of faeces were recorded for 15 weeks. Afterward, the mice were fasted, anesthetized with a Nembutal (pentobarbital) injection (0.75 μL/g body weight), and dissected. Blood was collected from the heart and mixed quickly with EDTA-2Na (ethylenediaminetetraacetic acid disodium salt, 2-hydrate) as an anticoagulant. Liver and kidneys were weighted, frozen in liquid nitrogen, and stored at −20 ℃ until analysis. Blood samples were centrifuged at 1000 ×*g* for 30 min to separate and the upper phase, i.e., the serum, was collected and stored at -85 ℃ until use for neutral lipid analysis and quantification

of cholesterol (total, HDL, and LDL), alanine aminotransaminase (ALT), and aspartate transaminase (AST). The analysis of the serum samples was conducted by Obihiro clinical laboratory, Inc. (Obihiro, Japan).

Liver and faecal lipid concentrations were measured according to a previously reported method [42]. In brief, liver and faecal lipids were extracted by a chloroform/methanol solution (2:1, v/v). The extracts were dried under N2 to constant weight and that weight value was recorded. Isopropanol was added, and liver and faecal cholesterol levels were determined by commercial kits (Wako Pure Chemical Industries, Ltd.).

The faecal bile acid content was measured as described previously [46]. Briefly, faeces were extracted by distilled water using sonication. The extract was centrifuged and the supernatants were collected. The bile acid content was determined from the supernatants using commercial kits (Wako Pure Chemical Industries, Ltd.).

Lipid peroxidation in liver was determined by measuring thiobarbituric acid-reactive substances (TBARS) with a slightly modified version of the protocol described in [46,47]. Briefly, 400 μL of 8.1% sodium dodecyl sulfate solution, 300 μL of 20% acetic acid (pH 3.5), and 500 μL of distilled water were successively added to 100 mg of liver. The mixture was homogenized using a Teflon homogenizer, and an equal volume of antioxidant solution (0.25 mg/mL copper sulfate) was added. After the mixture was incubated for 30 min at 37 °C, 950 μL was transferred to a capped test tube, to which 25 μL of 0.8% butylated hydroxytoluene acetic acid and 750 μL of 0.8% tertiary butyl alcohol were then added. The reaction mixture was placed for 1 h on ice, incubated at 100 ℃ for 1 h, and then cooled in running water. Afterward, 0.5 mL of distilled water and 2.5 mL of n-butanol/pyridine (15:1) were added, mixed vigorously, and centrifuged at 1,500 ×*g* for 10 min to obtain the supernatant, which was measured at 532 nm. Lipid peroxidation was expressed as the amount of produced 1,1,3,3-tetraethoxypropane.

2.8. Oral Starch Tolerance Test in Mice

After adaptation, the animals were randomly divided into experimental groups (8 mice per group). The mice were fasted for 24 h and blood was withdrawn from the tail vein and subjected to assays of blood glucose levels. SRPAs were suspended in physiological solution and used doses of 250 and 750 mg/kg. After the oral administration of the suspension of polyphenolic compounds for 30 min, a single oral injection of 2 g/kg starch in physiological solution was administered. Blood was withdrawn from the tail vein at 0.5, 1, and 2 h, and blood glucose levels were analyzed using the OMRON Precision Exceed HEA-216 (Omron Healthcare Co., Ltd., Kyoto, Japan) according to the manufacturer's instructions.

2.9. Statistical Analysis

Values are expressed as the mean ± standard error (S.E.M). Differences were evaluated by one-way ANOVA, followed by Tukey's studentized range (Honestly Significant Difference) test, using SAS v9.3 (SAS Institute Inc., Cary, NC, USA). Differences were considered significant when $p < 0.05$.

3. Results and Discussion

3.1. Polyphenol and Proanthocyanidin Content

The content of proanthocyanidin (179.51 mg/g seed coat) was 95% in polyphenols (188.72 mg/g seed coat) of 70% acetone extract of the scarlet runner beans' coat (Table 5). proanthocyanidin content was much higher than the reported content of pea, lentil, and faba bean (3.36, 3.29, and 6.54 mg/g seed, respectively) [17]. Moreover, we found proanthocyanidins was in the 70% acetone extract of the scarlet runner beans' coat by procyanidin qualitative analysis (Figure 6). We performed Sephadex LH-20 column

chromatography to obtain three fractions: Fra.I, Fra.II, and Fra.III. No proanthocyanidins were detected in Fra.I, whereas the proanthocyanidin content in Fra.II and Fra.III amounted to 47% and 53% of total weight, respectively (Figure 7). According to Saito et al. [38], Fra.I contains monomeric phenolic compounds; Fra.II, polyphenols with a low degree of polymerization; and Fra.III, polyphenols with a high degree of polymerization. Moreover, Fra.III exhibited higher DPPH radical scavenging activity than that of Fra.II. Fra.I was not detected DPPH radical scavenging activity (Figure 8).

3.2. Thiolysis Analysis of Fra.II and Fra.III

To investigate the composition and calculate the mDP values for Fra.II and Fra.III, thiolysis was carried out and the reaction products were analyzed by RP-HPLC (Figure 9). During the thiolytic cleavage of proanthocyanidins, terminal units are released as free flavan-3-ols, whereas all extension units are attacked by the nucleophilic agent (2-mercaptoethanol was used in this study) to generate the respective derivatives. We detected (+)-catechin and (−)-epicatechin monomers in Fra.II and Fra.III, indicating that these constituted the terminal units of the corresponding proanthocyanidins. Moreover, the (−)-epicatechin derivative was the compound with the largest peak area, suggesting that (−)-epicatechin was the main constituent compound for proanthocyanidins in both fractions. Regarding mDP, Fra.III had a higher value (9.6) than Fra.II (3.8). In both fractions, proanthocyanidins were of the B-type configuration. B-type proanthocyanidins were previously found in pea, lentil, and faba bean, and their mDPs was determined at 5–8 by thiolytic cleavage [17].

3.3. Inhibitory Effects of SRPAs on Lipase Activity in Vitro

Polyphenols from litchi flowers have been previously demonstrated to exert an inhibitory effect on porcine pancreatic lipase [48]. Generally, the suppressive effect of polyphenols on

lipase activity is attributed to their affinity for proteins, which leads to the aggregation of the enzyme [49]. The effects of SRPAs (total, Fra.II, and Fra.III) on pancreatic lipase activity were investigated. All three demonstrated a significant ($p < 0.05$) dose-dependent suppressive effect, with IC_{50} values of 4.12 ± 0.22, 3.88 ± 0.35, and 1.84 ± 0.46 μg/mL, respectively (Table 6). Since Fra.III contains proanthocyanidins with a higher degree of polymerization compared to those of Fra.II, the fact that the former had a stronger lipase inhibitory activity compared to the latter indicates that more highly polymerized proanthocyanidins have more potent inhibitory activities. As a means of comparison, we also analyzed the lipase inhibitory activity of epigallocatechin gallate, which has been reported to be a stronger lipase inhibitor than catechin, gallocatechin, and epicatechin. [50]. We calculated its IC_{50} value at 18.01 ± 1.39 μg/mL, thus it is a weaker lipase inhibitor than SRPAs.

3.4. Effects of SRPAs on Body Weight Gain, Organs, and Adipose Tissue in Mice Fed a High-Fat Diet

To assess the anti-obesity effects of SRPAs, mice were fed with different diets (see Subsection 1.6, “Animals and Diets”) for 15 weeks and various parameters were measured at the end of that period. Results are shown in Table 7 and 8. The mice of the HF group displayed the highest gain of body weight, whereas, as expected, SC mice exhibited the lowest gain of body weight. As made obvious by the results of the 1.0% SRPAHF, supplementation with SRPAs was clearly effective in attenuating the gain of body weight ($p < 0.05$ *vs.* the HF group), lowering it to a level close to the one seen in the SC group. A similar suppression of body weight gain was reported in studies investigating the effects of the consumption of polyphenols from green tea [51] and seaberry leaf [52]. In contrast, no significant difference was observed in our study among the different diet groups with respect to food consumption, water intake, and faecal weight.

We also investigated whether supplementation with SRPAs for 15 weeks affects the weight of organs and adipose tissue. Both 0.5% and 1.0% SRPAHF had lower liver and kidney mass, as well as less perirenal fat content, compared to the HF group. At 1.0% supplementation, SRPAs also suppressed the increasing effect of high-fat diet on peritesticular and periintestinal fat content, compared to the HF group. Similar decreases in adipose tissue weight have been reported for seaberry leaf polyphenols by Nishi et. al. [52]. The decrease in body weight gain by SRPAs might be responsible for the normalization of these organs and tissues [53].

Supplementation with 1.0% SRPAs significantly decreased liver lipid and cholesterol content. These results are in agreement with those of Ali et al. [54] who reported that rats fed a high-fat diet supplemented with cocoa polyphenols displayed decreased liver weight, as well as lower liver lipid and cholesterol content, compared with mice fed a high-fat diet only. We also observed that supplementation with 0.5 or 1.0% SRPAs significantly increased the lipid and total cholesterol content of faeces. Our results are similar to those of Uchiyama et al. [53], who reported that supplementation of high-fat diet with 5% black tea polyphenols increased faecal triglyceride content. We conclude that the inhibition of lipase activity by SRPAs reduced fat decomposition and, by extension, the absorption of free fatty acids. Thus, more fat was excreted, whereas less fat was accumulated.

3.5. Effects of SRPAs on Serum and Liver Lipid Peroxides in Mice Fed a High-Fat Diet

We also examined how the different diets affected various serum parameters associated with obesity including the levels of neutral lipids, cholesterol (total, HDL, and LDL), ALT, and AST. There were no significant changes in ALT and AST levels among the different diet groups.

In contrast, as seen in Table 9, both the 0.5% SRPAHF and the 1.0% SRPAHF group displayed significantly lower levels of neutral lipids and LDL cholesterol compared with the HF group. Moreover, supplementation with 1.0% SRPAs suppressed the increasing effect of high-fat diet on HDL cholesterol. Total cholesterol levels were significantly lower and the HDL:LDL ratio was significantly higher in the 1.0% SRPAHF group compared to the HF group. Similar decreases in total plasma cholesterol have been reported for proanthocyanidins isolated from the seed shells of Japanese horse chestnut [50] and black tea [53] and may be attributed to a decrease in the micellar solubility of cholesterol, as has been previously shown in a study using Reishi extracts [55]. In turn, the decrease in micellar solubility may result from specific interactions between proanthocyanidins and phosphatidylcholine, as was reported for epigallocatechin gallate [56].

To investigate possible changes in the liver antioxidant capacity, we analyzed liver lipid peroxide content by measuring the abundance of TBARS in the different diet groups. Both the 0.5% SRPAHF and the 1.0% SRPAHF group had significantly lower liver lipid peroxide levels compared with the HF group (Table 9), indicating that SRPAs increase the antioxidant capacity of the liver. This result is in agreement with that of a previous study in which supplementation of a high-fat diet with buckwheat miso decreased lipid peroxide levels compared with high-fat diet alone [46].

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3.6. Inhibitory Effects of SRPAs on Blood Glucose Levels after Oral Administration of Starch

We examined the inhibitory activities of SRPAs on α-amylase and α-glucosidase. SRPAs demonstrated dose-dependent suppressive effect α-amylase and α-glucosidase, with IC_{50} values of 15.08± 0.24, and 11.89 ± 0.28 μg/mL, respectively. SRPAs were tested for their inhibitory effects on the elevation of blood glucose levels by the oral starch tolerance test in mice. After the administration of starch, the maximum increase in the blood glucose level was observed at 30 min in all mice, but mice treated with 250 mg/kg and 750 mg/kg polyphenols exhibited significantly lower blood glucose concentrations than those of the control group ($p < 0.01$) (Figure 10A). At 60 min, the 250 mg/kg and 750 mg/kg SRPAs groups still showed significantly lower glucose levels than those of the control group ($p < 0.05$). At 120 min, the blood glucose concentrations recovered to the levels observed at 0 min. Polyphenols from kidney beans [57] and black beans [58] also reduce blood glucose in

rats. The area under the curve (AUC) of the blood glucose level over a 120-min period is shown in Figure 10B. The AUC value was significantly lower for the 750 mg/kg SRPAs group than for the other groups, and was significantly lower for the 250 mg/kg group than for the control group.

Table 5. Polyphenol and procyanidin content in SRPA.

	Polyphenols (mg/g seed coat)	Procyanidins (mg/g seed coat)
SRPA	188.72 ± 2.18	179.51 ± 3.46

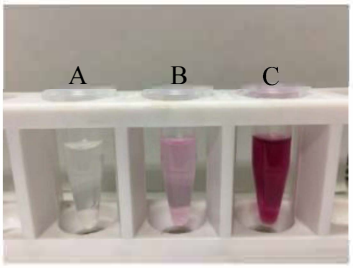

Figure 6. The procyanidin qualitative analysis for SRPA. Abbreviations: A; Blank (distilled water), B; Procyanidin B_2 (standard), C; SRPA.

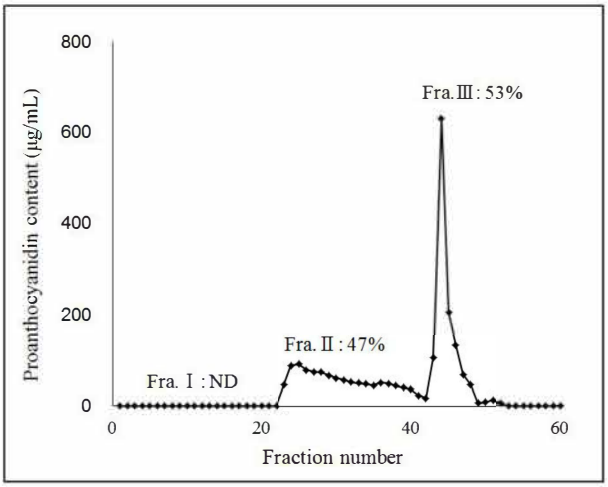

Figure 7. SRPA fraction was applied to an LH-20 column for the successive elution of Fra.I (ethanol fraction, 1–20); Fra.II (methanol fraction, 21–40), and; Fra.III (60% Acetone fraction, 41–60). ND, not detected.

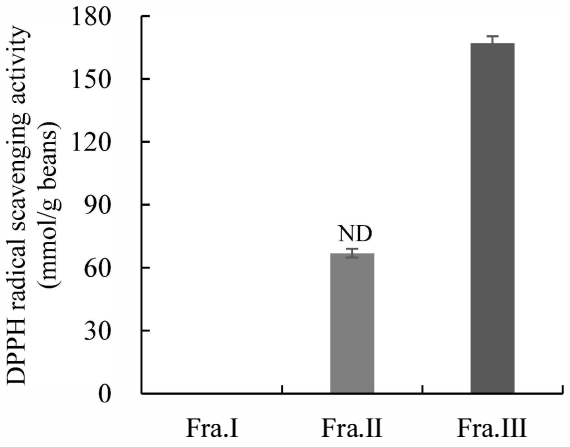

Figure 8. DPPH radical scavenging activity in Fra.I, Fra.II, Fra.III from SRPA. ND, not detected.

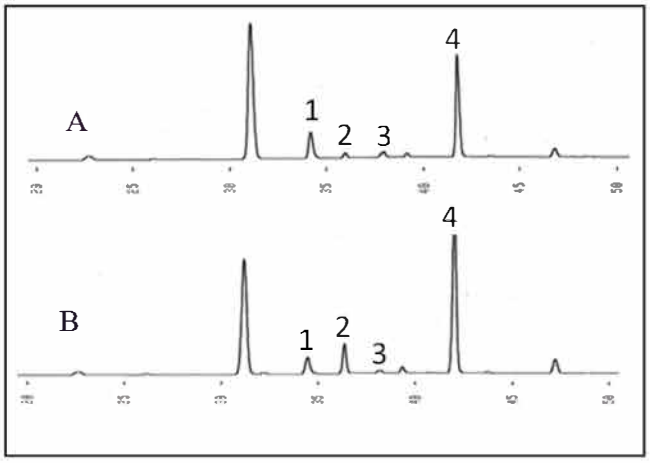

Figure 9. RP-HPLC chromatogram (280 nm) of the products of Fra.II (A) and Fra.III (B) thiolysis. Peaks: 1, (+)-catechin; 2, (+)-catechin derivative; 3, (−)-epicatechin; 4, (−)-epicatechin derivative.

Table 6. The lipase, α-amylase and α-glucosidase inhibitory activity of SRPA.

	Lipase inhibitory activity	α-Amylase inhibitory activity	α-Glucosidase inhibitory activity
	IC_{50} (μg/mL)	IC_{50} (μg/mL)	IC_{50} (μg/mL)
SRPA	4.12 ± 0.22[b]	15.08 ± 0.24	11.89 ± 0.28
Fra.I	ND	—	—
Fra.II	3.88 ± 0.35[b]	—	—
Fra.III	1.84 ± 0.46[c]	—	—
EGCG	18.01 ± 1.39[a]	—	—

Abbreviations: ND; not detected, —; not measure, EGCG; epigallocatechin gallate.

Table 7. Total body and organ weight measurements for the four dietary groups.

	HF	0.5% SRPAHF	1.0% SRPAHF	SC
Food consumption (g/day)	5.88 ± 0.42	5.67 ± 0.41	5.78 ± 0.58	6.03 ± 0.25
Water intake (mL/day)	6.45 ± 0.40	6.39 ± 0.47	6.40 ± 0.51	6.61 ± 0.35
Initial body weight (g)	42.97 ± 1.86	41.88 ± 2.97	42.33 ± 2.26	42.29 ± 3.28
Final body weight (g)	55.00 ± 2.88^{a}	$51.94 \pm 5.32^{a,b}$	$49.34 \pm 5.51^{a,b}$	47.43 ± 2.51^{b}
Body weight gain (g/15 weeks)	15.56 ± 7.35^{a}	$11.66 \pm 3.27^{a,b}$	9.52 ± 2.50^{b}	8.36 ± 1.64^{b}
Liver weight (g)	2.23 ± 0.20^{a}	1.83 ± 0.08^{b}	1.79 ± 0.12^{b}	1.75 ± 0.12^{b}
Liver lipid content (mg/g)	111.13 ± 7.25^{a}	111.52 ± 8.16^{a}	95.68 ± 7.11^{b}	80.55 ± 9.86^{c}
Liver cholesterol (mg/g)	7.86 ± 1.73^{a}	4.81 ± 0.92^{b}	5.15 ± 1.28^{b}	3.59 ± 0.59^{b}
Kidney weight (g)	0.85 ± 0.17^{a}	0.67 ± 0.05^{b}	0.67 ± 0.01^{b}	0.71 ± 0.03^{b}
Perirenal fat (g)	0.77 ± 0.16^{a}	0.49 ± 0.15^{b}	0.41 ± 0.16^{b}	0.45 ± 0.06^{b}
Periintestinal fat (g)	1.79 ± 0.47^{a}	$1.34 \pm 0.36^{a,b}$	$1.06 \pm 0.33^{b,c}$	0.71 ± 0.16^{c}
Peritesticular fat (g)	2.71 ± 0.37^{a}	$2.29 \pm 0.84^{a,b}$	1.75 ± 0.79^{b}	1.57 ± 0.36^{b}

Values represent mean ± S.E.M. In each column, differences between values superscripted with different letters were significant ($p < 0.05$).

Table 8. Faecal weight and lipid content measurements for the four dietary groups.

	HF	0.5% SRPAHF	1.0% SRPAHF	SC
Faecal weight (g/day)	1.06 ± 0.08	1.05 ± 0.07	1.16 ± 0.02	1.13 ± 0.01
Faecal lipid content (mg/day)	83.99 ± 19.35^{b}	78.84 ± 9.12^{b}	103.46 ± 11.35^{a}	102.88±11.68^{a}
Faecal cholesterol (mg/day)	3.43 ± 0.57^{c}	4.22 ± 0.44^{c}	5.37 ± 1.13^{b}	7.94 ± 0.97^{a}
Faecal bile acid (μmol/day)	0.28 ± 0.05	0.31 ± 0.08	0.30 ± 0.05	0.32 ± 0.12

Values represent mean ± S.E.M. In each column, differences between values superscripted with different letters were significant ($p < 0.05$).

Table 9. Serum and liver lipid peroxide measurements for the four dietary groups.

	HF	0.5% SRPAHF	1.0% SRPAHF	SC
Netural lipids (mg/dL)	111.00 ± 30.76^{a}	63.60 ± 30.62^{b}	47.40 ± 15.18^{b}	41.80 ± 12.11^{b}
Total cholesterol (mg/dL)	194.20 ± 27.43^{a}	$152.40 \pm 38.28^{a,b}$	139.20 ± 20.90^{b}	137.40 ± 11.22^{b}
HDL cholesterol (mg/dL)	141.60 ± 26.60^{a}	$118.60 \pm 25.69^{a,b}$	110.00 ± 17.58^{b}	109.20 ± 4.66^{b}
LDL cholesterol (mg/dL)	31.60 ± 5.90^{a}	22.00 ± 7.31^{b}	$17.00 \pm 4.64^{b,c}$	12.00 ± 2.92^{c}
HDL:LDL	4.47 ± 0.55^{c}	$5.71 \pm 0.82^{b,c}$	6.73 ± 1.48^{b}	8.76 ± 1.38^{a}
AST (U/L)	187.2 ± 43.24	143.40 ± 52.63	141.00 ± 47.37	138.40 ± 18.12
ALT (U/L)	25.60 ± 3.85	23.60 ± 6.11	23.60 ± 5.90	31.00 ± 8.89
Liver lipid peroxides (nmol/g)	265.34 ± 14.90^{a}	220.37 ± 39.20^{b}	203.86 ± 22.70^{b}	196.77 ± 17.05^{b}

Values represent mean ± S.E.M. In each column, differences between values superscripted with different letters were significant ($p < 0.05$).

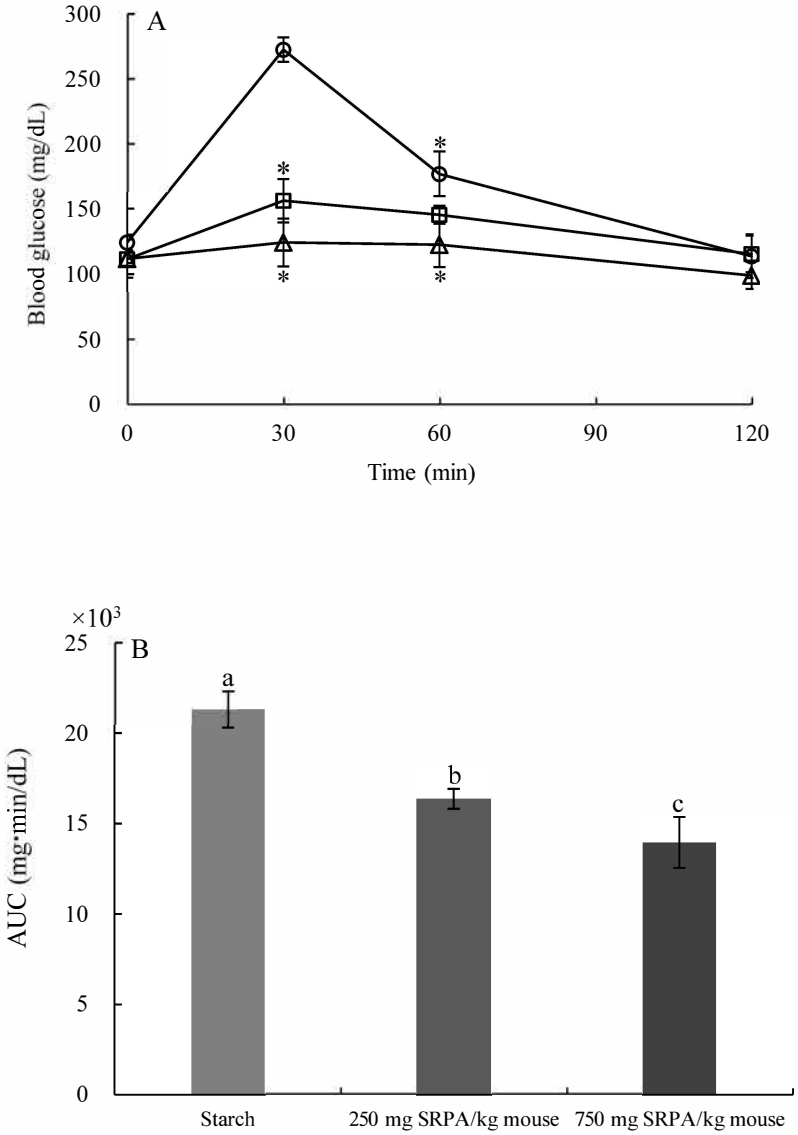

Figure 10. Oral starch tolerance test to monitor the inhibitory effect of SRPAs on blood glucose levels in mice (A) and AUC values (B). Values are presented as the means ± standard error of 5 mice per group. * indicates a significant difference with respect to the control (*$p < 0.05$). Symbols: ○, 0 mg/kg; □, 250 mg/kg; △, 750 mg/kg mouse. Values followed by different letters in a column are significantly different ($p < 0.05$).

IV. The Effect of Polyphenols from Scarlet Runner Beans (Purple) by Thermal Processing

1. Introduction

Legume must be processed prior to consumption (such as germinating, roasting, soaking and cooking) in order to improve their palatability and nutritional quality, and these processes produce changes in their nutritional composition and content of bioactive compounds [23] [24]. In this section, the effect of boiling pressure on the polyphenol compounds and the functionality in purple scarlet runner beans were investigated.

2. Materials and Methods

2.1. Materials

The materials of scarlet runner beans (purple), all other reagents and chemicals were purchased from Kawanishi Agricultural Cooperative Association (Obihiro, Japan) and Wako Pure Chemical Industries, Ltd. (Osaka, Japan) (see Subsection III, “Materials”), respectively, unless stated otherwise.

2.2. Thermal Processing

Approximately 30g of scarlet runner beans (purple) were added with 120 mL of distilled water, and soaked for 18 h at 28°C. We added soaked liquid with edible brewed vinegar at 0.1% and 0.5%, and edible baking soda at 200 mg and 400 mg. In general, in order to make cooked beans be soft and easy to eat, 30 g raw beans need 200 mg baking soda. The brewed vinegar was purchased from Mizkan Holdings Co., Ltd (Hanga, Japan), and the acidity was 4.2%. The baking soda was a kind of food additive, and purchased from Kouta Shouten (Osaka, Japan). The soaked liquid adding with edible brewed vinegar or baking soda, and

soaked beans were cooked with pressure cooker (Panasonic Co., Ltd., Osaka, Japan) at 113°C, low pressure for 7 min. Cooked beans and cooking liquid were separated for analysis (Table 10. The effect on pH adding with vinegar and baking soda was showed in Table 11. The pH meter D-15 was obtained from Horiba (Kyoto, Japan).

2.3. Cooked Beans Extract Preparation and Treatment of NaOH Solution for Extraction Residues

The cooked bean extraction residues were dried with drying machine. 1 g of the dried residues were added 0.1N NaOH solution and ultrasound treatment for 30 min, the mixture was centrifuged at 1,006 × *g* for 10 min to obtain a supernatant. The same extraction process was repeated three more times. The supernatants were neutralized with 1N acetate and added distilled water and hexane. The mixture was centrifuged at 1,006 × *g* for 10 min to obtain a supernatant (aqueous layer). The aqueous layer was for quantified analysis of polyphenols and DPPH radical scavenging activity (see Subsection III, “Materials”).

2.4. Statistical Analysis

Values are presented as means ± standard error. Statistical significance was evaluated by ANOVA and least significant difference (LSD) tests (SAS Enterprise Guide 5.1). Differences were considered significant when $p < 0.05$.

3. Results and Discussion

3.1. Polyphenols in cooked bean extracts and cooking liquid

Scarlet runner beans were processed by pressure cooker, cooked beans and cooking liquid were obtained. The cooked beans were homogenized and extracted by 80% ethanol and 70% acetone, and the extract residues were treated with 0.1N NaOH solution. Table 1 showed

polyphenol content in cooked bean extracts, cooked bean extraction residues with the treatment of 0.1N NaOH solution and cooking liquid.

Comparing with raw beans, the total polyphenol content was decreased by 31.12% (Table 12). Xu et al., 2011 [59] reported that the polyphenols was decreased by thermal treatment in pinto beans, black beans and black soybeans, however, was increased in yellow soybeans.

The total polyphenol content was 7.56 mg/g raw bean in control, 8.63 mg/g raw bean in 0.1% vinegar and 8.89 mg/g raw bean in 0.5% vinegar (Table 12). The total polyphenol content was increased by 14.15% and 17.59%, respectively, adding with 0.1% and 0.5% brewed vinegar. However, they are significant lower than that of raw beans. The total polyphenol content was decreased by 9.92% and 8.20%, respectively, adding with 200 mg and 400 mg baking soda. The effect of polyphenol content may be a polyphenols in beans decomposition and recomposition after adding with brewed vinegar and baking soda.

Moreover, cooked beans extract residues were treated with 0.1N NaOH solution. Polyphenols were found in the cooked beans extract residues (Table 12). The polyphenol content in control was significant lower than brewed vinegar, and significant higher than baking soda. In other words, 80% ethanol and 70% acetone couldn't extract polyphenols adequately. Proportion of starch is approximately 90% in beans. According to Kitahara et al., 1997 [60] reported that the starch could absorb polyphenol, we speculate that it could lead to extract process of polyphenols in cooked beans become difficult. NaOH solution treatment of cooked beans could extract more polyphenols than those of 80% ethanol and 70% acetone. However, NaOH solution treatment may be result in polyphenols decomposition, it need more investigation.

3.2. DPPH radical scavenging activity in cooked beans and cooking liquid

Table 13 showed DPPH radical scavenging activity in cooked beans and cooking liquid. DPPH radical scavenging activity was not detected in treatment with 0.1N NaOH solution.

Comparing with raw beans, the total DPPH radical scavenging activity was decreased by 66.54%. DPPH radical scavenging activity was 23.44 μmol/g raw bean in control, 18.20 μmol/g raw bean in 0.1% vinegar and 15.07μmol/g raw bean in 0.5% vinegar. DPPH radical scavenging activity was increased by 29.69% and 27.35%, respectively, adding with 0.1% and 0.5% brewed vinegar. However, they are significant lower than that of raw beans. DPPH radical scavenging activity was increased by 30.46% and 27.77%, respectively, adding with 200 mg and 400 mg baking soda.

Table 10. The effect on cooked beans and cooking liquid adding with vinegar and baking soda.

	Raw beans (g)	Soaked liquid (mL)	Soaked beans (g)	Cooked beans (g)	Cooking liquid (mL)
0.1% Vinegar	30.05	67.72	83.0	77.57	53.00
0.5% Vinegar	30.49	67.85	82.0	76.63	57.00
Control	30.45	69.24	82.0	81.71	50.00
200 mg Baking soda	30.13	67.96	81.5	79.00	46.00
400 mg Baking soda	30.95	68.08	83.0	81.97	38.00

Table 11. The effect on pH adding with vinegar and baking soda.

	pH		
	Soaked liquid	Soaked liquid after addition	Cooking liquid
0.1% Vinegar	6.08	4.34	5.18
0.5% Vinegar	5.96	3.59	4.47
Control	6.04	—	6.08
200 mg Baking soda	5.85	7.10	7.42
400 mg Baking soda	5.99	7.53	8.15

Table 12. Polyphenol content in cooked bean extracts, cooked bean extract residues with the treatment of 0.1N NaOH solution and cooking liquid.

	Polyphenol content (mg/g raw bean)					
	0.1% Vinegar	0.5% Vinegar	Control	200 mg Baking soda	400 mg Baking soda	Raw beans
Cooked beans*	$2.43^{a} \pm 0.04$	$2.29^{b} \pm 0.01$	$2.33^{e} \pm 0.09$	$1.93^{d} \pm 0.03$	$2.21^{c} \pm 0.05$	—
Cooked beans**	$2.51^{b} \pm 0.01$	$2.81^{a} \pm 0.02$	$2.19^{c} \pm 0.02$	$1.2^{d} \pm 0.01$	$0.94^{e} \pm 0.01$	—
Cooked beans***	$4.94^{a} \pm 0.05$	$5.10^{a} \pm 0.02$	$4.52^{b} \pm 0.08$	$3.13^{c} \pm 0.03$	$3.15^{c} \pm 0.06$	—
Cooking liquid	$3.68^{a} \pm 0.41$	$3.79^{a} \pm 0.18$	$3.03^{b} \pm 0.21$	$3.72^{a} \pm 0.03$	$3.33^{a} \pm 0.03$	—
Total	$8.63^{b} \pm 0.43$	$8.89^{b} \pm 0.18$	$7.56^{c} \pm 0.30$	$6.81^{d} \pm 0.40$	$6.94^{d} \pm 0.20$	$10.98^{a} \pm 0.03$

* Polyphenols in cooked beans extracts, ** Polyphenols in cooked bean extraction residues with the treatment of 0.1N NaOH solution,

*** The total polyphenols in cooked beans. Values within a column followed by different letters are significant at $p < 0.05$.

Table 13. DPPH radical scavenging activity in cooked beans and cooking liquid.

	DPPH radical scavenging activity (μmol/g raw bean)					
	0.1% Vinegar	0.5% Vinegar	Control	200 mg Baking soda	400 mg Baking soda	Raw beans
Cooked beans	$18.20^{a} \pm 0.41$	$15.07^{b} \pm 0.28$	$13.99^{c} \pm 0.27$	$10.00^{e} \pm 0.48$	$11.45^{d} \pm 0.24$	—
Cooking liquid	$12.20^{d} \pm 2.70$	$14.78^{c} \pm 1.35$	$9.44^{e} \pm 0.51$	$20.58^{a} \pm 0.74$	$18.49^{b} \pm 0.76$	—
Total	$30.40^{b} \pm 0.82$	$29.85^{b} \pm 0.84$	$23.44^{c} \pm 0.42$	$30.58^{b} \pm 1.17$	$29.95^{b} \pm 0.84$	$46.17^{a} \pm 0.66$

Values within a column followed by different letters are significant at $p < 0.05$.

V. Conclusions

Scarlet runner beans contained polyphenols, antioxidant activity and α-glucosidase inhibitory activity. The seed coat of scarlet runner beans (purple) polyphenols exhibited anti-obesity effects and the suppression of blood glucose levels through the inhibition of lipase, α-amylase and α-glucosidase. The seed coat of scarlet runner beans (purple) polyphenols contained mainly polymerized proanthocyanidins, and their mDP values were 3.8-9.6. Moreover, the adding of edible brewed vinegar during scarlet runner beans (purple) thermal process could increase the polyphenols and their antioxidant activity of cooked beans.

VI. Abstract

Scarlet runner beans are a valuable source of many nutrients, including proteins, starch, dietary fiber, and oligosaccharides, and are used in various foods in Japan. To extend our knowledge of the effects of scarlet runner beans on human health, we analyzed the color, polyphenol and procyanidin content, DPPH radical scavenging activity, reducing power, and α-glucosidase inhibitory activity of various scarlet runner beans. The L^* and C values were the highest for scarlet runner beans (white) and lowest for scarlet runner beans (black). Scarlet runner beans (purple) and scarlet runner beans (brown) showed significant higher polyphenol and procyanidin content than those of scarlet runner beans (white) and scarlet runner beans (mixed).

Scarlet runner beans (brown) and scarlet runner beans (mixed) showed the highest DPPH radical scavenging activity and reducing power. Scarlet runner beans (white) had the lowest ratio of oligomeric and polymeric polyphenols and the lowest DPPH radical scavenging activity and reducing power. We found a positive correlation between polyphenol content and both DPPH radical scavenging activity and reducing power. Moreover, polyphenols from various scarlet runner beans inhibited the activity of α-glucosidase in a dose-dependent manner. The polyphenols (50 μg/mL) of scarlet runner beans (black) showed the highest α-glucosidase inhibitory activity (85.7%), and those of scarlet runner beans (white) showed the lowest inhibitory activity (53.8%). Scarlet runner beans (black) had a lower IC_{50} value (26.4 μg/mL) and scarlet runner beans (white) had a higher IC_{50} value (58.4 μg/mL) than those of other scarlet runner beans.

Proanthocyanidins are oligomeric or polymeric flavonoids found in several plants. Polyphenol content was 188.72 mg/g seed coat of scarlet runner beans (purple), and those of proanthocyanidin content was 179.51 mg/g seed coat. The proportion of proanthocyanidins were 95% in polyphenols of scarlet runner beans (purple) seed coat. The seed coat of scarlet

runner beans (purple) contained mainly oligomeric and polymeric polyphenols, and their proportions were 47% and 53%, respectively. Moreover, polymeric polyphenols exhibited higher DPPH radical scavenging activity than that of oligomeric polyphenols. Oligomeric and polymeric polyphenols demonstrated a significant ($p < 0.05$) dose-dependent suppressive effect on lipase, with IC_{50} values of 3.88 ± 0.35, and 1.84 ± 0.46 μg/mL, respectively. These results indicates that more highly polymerized polyphenols exhibited more potent DPPH radical scavenging activity and lipase inhibitory activity.

To investigate the composition and calculate the mDP values for oligomeric and polymeric polyphenols, thiolysis was carried out and the reaction products were analyzed by RP-HPLC. We detected (+)-catechin and (−)-epicatechin monomers in reaction products of oligomeric and polymeric polyphenols fractions, indicating that these constituted the terminal units of the corresponding proanthocyanidins. Moreover, the (−)-epicatechin derivative was the compound with the largest peak area, suggesting that (−)-epicatechin was the main constituent compound for proanthocyanidins in both fractions. Regarding mDP, polymeric polyphenols had a higher value (9.6) than oligomeric polyphenols (3.8). In both fractions, polyphenols were of the B-type configuration.

Moreover, we examined polyphenols from seed coat of scarlet runner beans (purple) (SRPA) effect on mice fed a high-fat diet. Male mice were assigned to the following seven-subject dietary groups: (1) high-fat diet (HF); (2) high-fat diet supplemented with 0.5% SRPA (0.5% SRPAHF); (3) high-fat diet supplemented with 1.0% SRPA (1.0% SRPAHF), and; (4) standard chow (SW) for 15 weeks. SRPA supplementation decreased body weight gain; liver and kidney weight; liver, perirenal, peritesticular, and periintestinal fat content; liver cholesterol level, and; serum neutral lipid and cholesterol levels, whereas the fecal lipid content and the liver anti-oxidative capacity were increased. These findings suggest a potential use of SRPAs as a dietary supplement exerting anti-obesity effects through the

inhibition of fat digestive enzymes.

Moreover, we examined the inhibitory activities of SRPAs on α-amylase and α-glucosidase. SRPAs demonstrated dose-dependent suppressive effect α-amylase and α-glucosidase, with IC_{50} values of 15.08± 0.24, and 11.89 ± 0.28 μg/mL, respectively. SRPAs were tested for their inhibitory effects on the elevation of blood glucose levels by the oral starch tolerance test in mice. After the administration of starch, the maximum increase in the blood glucose level was observed at 30 min in all mice. Comparing with control group, mice treated with 250 mg/kg and 750 mg/kg SRPAs exhibited significantly lower blood glucose concentrations ($p < 0.05$). At 60 min, the 250 mg/kg and 750 mg/kg SRPAs groups still showed significantly lower glucose levels than those of the control group ($p < 0.05$). At 120 min, the blood glucose concentrations of all groups recovered to the levels observed at 0 min. These results suggest that SRPAs effectively suppressed the elevation of blood glucose levels after the oral administration of starch in mice. The suppression of blood glucose levels through the inhibition of starch and disaccharide digestive enzymes.

Scarlet runner beans (purple) were processed by pressure cooker, cooked beans and cooking liquid were obtained. Moreover, we added edible brewed vinegar and baking soda during thermal process. Comparing with raw beans, the total polyphenol content (the total polyphenol content value of cooked beans and cooking liquid) and total DPPH radical scavenging activity (the total DPPH radical scavenging activity value of cooked beans and cooking liquid) were decreased by 31.12% and 66.54%, respectively. The total polyphenol content was increased by 14.15% and 17.59%, respectively, adding with 0.1% and 0.5% brewed vinegar. However, they are significant lower than that of raw beans. The total polyphenol content was decreased by 9.92% and 8.20%, respectively, adding with 200 mg and 400 mg baking soda. The effect of polyphenol content may be a polyphenols in beans decomposition and recomposition after adding with brewed vinegar and baking soda. DPPH

radical scavenging activity was increased by 29.69% and 27.35%, respectively, adding with 0.1% and 0.5% brewed vinegar. However, they are significant lower than that of raw beans. DPPH radical scavenging activity was increased by 30.46% and 27.77%, respectively, adding with 200 mg and 400 mg baking soda. Moreover, cooked beans extract residues were treated with 0.1N NaOH solution. Polyphenols were from 0.94 mg/g raw bean to 2.81 mg/g raw bean in cooked beans extract residues of various manufacturing process treatment.

These findings suggest that polyphenols from the seed coat of scarlet runner beans (purple) as a dietary supplement exerting anti-obesity effects through the inhibition of fat digestive enzymes and the suppression of blood glucose levels through the inhibition of starch and disaccharide digestive enzymes. Moreover, the adding of edible brewed vinegar during scarlet runner beans (purple) thermal process could increase the polyphenols and their antioxidant activity of cooked beans.

要約

種皮色の異なる 5 品種の紫、茶、混ぜ、黒、白花豆を調べたところに、白花豆以外の花豆には豊富なポリフェノール、プロシアニジン、オリゴマー型及びポリマー型ポリフェノールが含まれ、強い DPPH ラジカル消去活性と還元力が認められた。特に、紫花豆のポリフェノール含量と抗酸化活性が有意に高いことを明らかにした。他の花豆と比べ、白花豆のポリフェノール含量とプロシアニジン含量、オリゴマー型とポリマー型ポリフェノールの割合、DPPH ラジカル消去活性と還元力は低かった。これらのことから、ポリフェノール含量とポリフェノールの重合度が共に抗酸化活性に影響のあることが明らかにした。5 品種花豆に含まれるポリフェノール含量と DPPH ラジカル消去活性と還元力との間には、それぞれ高い正の相関性が認められ、相関係数 R^2 はそれぞれ 0.96 と 0.97 であった。また、5 品種花豆ポリフェノールは α-グルコシダーゼ活性を

用量依存的に阻害することを明らかにした。特に、黒花豆と紫花豆ポリフェノールの IC_{50} 値は 26.4 μg/ mL と 39.7 μg/ mL で、強い α-グルコシダーゼ阻害活性が認められた。また、黒花豆ポリフェノールは α-グルコシダーゼに対して、非拮抗阻害の様式であることを明らかにした。

花豆の種皮と子葉のポリフェノール含量は、種皮に含まれるポリフェノール含量の方が子葉のそれらよりも顕著に多いことを明らかにした。紫花豆皮ポリフェノール（SRPA）は 95%以上がプロシアニジンであり、オリゴマー型（Fra.II）とポリマー型のポリフェノール（Fra.III）がそれぞれ 47%と 53%の割合であった。SRPA プロシアニジンの主な構造は(−)-エピカテキンの B 型結合の重合体であり、オリゴマー型ポリフェノールの平均重合度は 3.8、ポリマー型ポリフェノールのそれは 9.6 であった。また、SRPA、Fra.II及び Fra.IIIはリパーゼ活性を用量依存的に阻害することを明らかにした。リパーゼ活性阻害の強い順に、Fra.III、Fra.II、SRPA で、いずれも、エピガロカテキンガレート（標品）のリパーゼ活性阻害よりも強いことを明らかにした。また、Fra.IIIの DPPH ラジカル消去活性は Fra.IIのそれよりも強かった。これらのことから、紫花豆皮ポリフェノールのリパーゼ活性阻害と DPPH ラジカル消去活性はポリフェノールの重合度に関係があり、重合度が高いポリフェノールが強いリパーゼ活性阻害と DPPH ラジカル消去活性を示すことを明らかにした。

SRPA の高脂肪食マウスに対する抗肥満効果について検討した。SRPA は高脂肪食マウスの体重上昇を抑制し、血清の中性脂質量及び肝臓の脂質含量が有意に低いこと、血清コレステロール上昇の抑制、HDL と LDL の比は有意に高いことが認められた。また、SRPA 投与群マウスの糞中脂質含量と糞中コレステロール濃度が有意に高いことが認められた。SRPA と高脂肪食を

与えたマウス肝臓の脂質含量、コレステロール含量及び過酸化脂質含量のいずれも低いことが認められた。これらのことから、SRPA は *in vivo* において、抗肥満効果を示すこと、肝臓の過酸化脂質量が減少していたことから、抗酸化活性効果のあることが認められた。また、SRPA はリパーゼ活性阻害が認められ、糞への脂肪排泄が多かったことから、脂肪の分解· 吸収の抑制、体内脂肪蓄積の抑制が起きたと考えられる。

In vitro において、SRPA は α-アミラーゼ活性及び α-グルコシダーゼ活性を用量依存的に阻害することを明らかにした。SRPA をマウスに経口投与して血糖値上昇抑制作用を検討したところ、SRPA 投与群のマウス血中グルコース含量は、コントロール群のそれに比べて有意に低い値を示した。これらのことから、SRPA は α-アミラーゼ及び α-グルコシダーゼ活性を阻害することにより、マウスの血糖値上昇を抑制したと推定した。

以上のことから、SRPA はマウスのリパーゼ活性、α-アミラーゼ活性及び α-グルコシダーゼ活性を阻害することにより、マウスの抗肥満及び血糖値上昇抑制作用を示すことを明らかにした。SRPA の主な成分は、平均重合度が３～10 のプロシアニジンであることを明らかにした。

紫花豆を加熱した時のポリフェノールと DPPH ラジカル消去活性に及ぼす影響を検討した。煮豆と煮汁ポリフェノールの合計値は生豆に比べ 31.1％減少し、DPPH ラジカル消去活性は 66.5%減少した。また、煮豆の製造工程に 0.1%と 0.5%の食酢を添加することにより、無添加のコントロールに比べ、ポリフェノール含量は 17.6%、DPPH ラジカル消去活性は 29.7％増加した。これらのことから、煮豆を製造する時には 0.1%程度の食酢を添加することにより、煮豆のポリフェノール含量を増大させられることを明らかにした。

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