

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

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Abstract Scarlet runner beans (SRB) 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 SRB on human health, we analyzed the color, polyphenol and procyanidin contents, DPPH radical scavenging activity, and reducing power of various SRB. The L* and C values were highest for SRB (white) and lowest for SRB (black). SRB (purple) and SRB (brown) showed higher polyphenol and procyanidin contents 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) 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 SRB inhibited the activity of α -glucosidase in a dose-dependent manner. The polyphenols (50 μ g/mL) of SRB (black) showed the highest α -glucosidase inhibitory activity (85.7%), and those of SRB (white) showed the lowest inhibitory activity (53.8%). SRB (black) had a lower IC₅₀ value (26.4 μ g/mL) and SRB (white) had a higher IC₅₀ value (58.4 μ g/mL) than those of other SRB. We speculate that the degree of polymerization for polyphenols affects antioxidant activity and α -glucosidase inhibitory activity for SRB. These results suggest that SRB has antioxidant and enzyme inhibitory effects via its polyphenols and provide a basis for selecting SRB cultivars and for developing SRB-based functional foods with improved health benefits.

Keywords: scarlet runner bean, polyphenol, DPPH radical scavenging activity, reducing power, α -glucosidase inhibitory activity

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1. Introduction

Free radicals cause oxidative damage, which is associated with several chronic human diseases, including cardiovascular diseases, neural disorders, such as Alzheimer's and Parkinson's disease, diabetes, and cancer [1]. Plant-derived phenolic compounds have been identified as antioxidants; they can delay or inhibit oxidative damage, thus preventing the onset of oxidative stress-related diseases in humans [2]. In addition to antioxidant activity, phenolic compounds play a key role in the inhibition of α -glucosidase, an intestinal cell membrane enzyme that can hydrolyze polysaccharides. Hence, inhibiting α -glucosidase activity may be an effective way to treat pre-diabetes and slow the progression of diabetes [3]. Many studies have reported that phenolic compounds from plants have α -glucosidase inhibitory activity [4,5,6].

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 [7] and *Phaseolus angularis* beans [8]. Purple scarlet runner beans (SRB, purple) are a potentially useful dietary supplement with anti-obesity effects via the inhibition of fat digestive enzymes [9]. Scarlet runner bean (*Phaseolus coccineus* L.) is cultivated for its seeds (dried or fresh), but is also an ornamental plant [10]. The dry seeds are used in salads, soups, and amanatto in Japan.

In the present study, we examined the antioxidant activity and α -glucosidase inhibitory activity of polyphenols from scarlet runner beans.

2. Materials and Methods

2.1. Materials

Samples of 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. The weight of 100 grains of SRB ranged from 175.2 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.

2.2. 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:

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

2.3. 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 $1,006 \times g$ 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.

2.4. Quantification of Polyphenols

Polyphenols were quantified using the Folin–Ciocalteu method [11]. The methanol fraction (HP-20 column) (100 μ L) was treated with 300 μ L of distilled water, 400 μ L of Folin–Ciocalteu reagent, and 400 μ L of a 10% Na_2CO_3 solution. The mixture was prepared in triplicate, incubated at 30°C for 30 min, and centrifuged at $1,006 \times g$ 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).

2.5. Quantification of Procyanidins

The procyanidin content of the methanol fraction (HP-20 column) was determined by the HCl-butanol method [12] using cyanidin as the standard equivalent.

2.6. Estimation of DPPH Radical Scavenging Activity

DPPH radical scavenging activity was evaluated by the method described by Brand-Williams et al. [13], with some modifications. A 50- μ L aliquot of the methanol fraction (HP-20 column) was mixed with 100 μ L of ethanol, and the mixture was supplemented with 150 μ L 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 μ mol trolox equivalents per gram of beans (μ mol/g).

2.7. Estimation of Reducing Power

Reducing power was evaluated according to a previously reported method, with minor modifications [14]. Briefly, 250 μ L of the methanol fraction (HP-20 column) was mixed with 250 μ L of sodium phosphate buffer (pH 7.5) in a test tube, and 250 μ L of 1% (w/v) potassium ferricyanide was added. The mixture was incubated at 50°C for 20 min. After the incubation period, 250 μ L of 10% trichloroacetic acid was added and centrifuged at $1,006 \times g$ for 10 min. The upper layer of the supernatant (500 μ L) was mixed with 500 μ L of distilled water and 100 μ L 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).

2.8. α -Glucosidase Inhibitory Activity

α -Glucosidase inhibition was analyzed following the methods of Matsumoto et al. [15], 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 μ L of 0.4% sucrose, 625 μ L of 0.1 mol/L sodium phosphate buffer (pH 6.8), and 125 μ L 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 μ g/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 μ L 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 IC_{50} value.

2.9. Statistical Analysis

Values are presented as means \pm 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. 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. [16], 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 [17]. The C value was highest for SRB (white) (8.8) and lowest for SRB (black) (0.4).

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

| | 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 ($p < 0.05$).

3.2. 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. [16]. 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 [16]. 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). Many researchers have previously found a positive correlation between the polyphenol content and DPPH radical scavenging activity for the pods of common beans [18], legumes in India [19], and thirteen genotypes of faba beans [20].

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

| | Polyphenols (mg/g) | Procyanin (mg/g) | DPPH radical scavenging activity ($\mu\text{mol/g}$) | 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 ($p < 0.05$).

3.3. 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 (Table 3). According to Saito et al. [21], 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%). 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 [18]. Moreover, we analyzed Fra.II and Fra.III by MALDI-TOF/MS. SRB (white) contained catechin and gallic acid 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. [4] detected gallic acid as a basic unit in polymeric polyphenols of okra seeds by MALDI-TOF/MS.

Table 3. Sephadex LH-20 column chromatogram of polyphenols prepared in scarlet runner beans. 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

| | 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.

3.4. 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 $\mu\text{g/mL}$) 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 $\mu\text{g/mL}$), and SRB (white) had a higher IC_{50} value (58.4 $\mu\text{g/mL}$) those of other SRB. Phenolic compounds from seven legumes [21] and soybeans [22] 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 [23]. Based on the α -glucosidase inhibitory activity of other SRB in vitro, we speculate that they also had the potential to reduce blood glucose.

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

| | Inhibitory activity of 50 $\mu\text{g/mL}$ polyphenols | IC_{50} |
|--------------|---|----------------------|
| | (%) | ($\mu\text{g/mL}$) |
| 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 ($p < 0.05$).

4. Conclusions

SRB (except white) showed high polyphenol contents, procyanidin contents, DPPH radical scavenging activity, and reducing power. SRB (white) had a relatively lower ratio of oligomeric and polymeric polyphenols, and lower antioxidant activity and α -glucosidase inhibitory activity than those of other SRB. It is considered the degree of polymerization for polyphenols affects antioxidant activity and α -glucosidase inhibitory activity. These observations indicate that SRB has useful properties and, in particular, has potential applications as an antioxidant and for the treatment of diabetes.

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Statement of Competing Interests

The authors have no competing interests.

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