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The Functionalities of Cooked Bean and Cooking Liquid Polyphenols from Red Kidney Bean

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赤金時豆煮豆及び煮汁ポリフェノールとその機能性について

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Abstract

Red kidney beans (RKB) were thermal processing by pressure cooker, cooked beans (RKB-CB) and cooking liquid (RKB-CL) were obtained. The extraction was purified by HP-20 column, polyphenols were 203.79 mg and 61.66 mg in RKB-CB and RKB-CL from 100 g RKB. RKB-CB had mostly monomeric polyphenols, and RKB-CL had high ratio of oligomeric polyphenols. The DPPH radical scavenging activity was 61.34 mmol and 20.73 mmol in RKB-CB and RKB-CL from 100 g RKB, the former showed significantly higher DPPH radical scavenging activity. Moreover, polyphenols from RKB-CL had strong α-glucosidase inhibitory activity (IC_{50} value, 6.0 μg/mL), and extremely weak inhibitory activity was found in RKB-CB (>80 μg/mL). These results suggest that polyphenol content and antioxidant activity from RKB-CB are greater than those of RKB-CL, and polyphenols from RKB-CB exhibit weaker α-glucosidase inhibitory activity than that of RKB-CL. The polyphenol fraction was applied to Sephadex LH-20 column, and eluted with Fra.I, Fra.II and Fra.III. Comparing with Fra.I, Fra.II of RKB-CL mainly inhibited α-glucosidase activity, so thus we conclude α-glucosidase inhibitory activity was related to oligomeric polyphenols.

Keywords: kidney bean, polyphenol, DPPH radical scavenging activity, α-glucosidase inhibitory activity

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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 (Xu et al. 2007). 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 (Willett 1994). 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 (Baron 1998). Lu et al. (2016), Dong et al. (2012) and Shobana et al. (2009) have reported that phenolic compounds from plants have α-glucosidase inhibitory activity.

Legumes are important food sources for humans in many developing countries. In addition to protein, carbohydrates (dietary fiber), minerals, and vitamins, they also contain a wide range of phytochemicals, including phenolics with antioxidant and other bioactivities. Anti-inflammatory activities of phenolic compounds have been detected in raw (unprocessed) red and white kidney beans (Garcia-Lafuente et al. 2014). Kidney beans (*Phaseolus vulgaris* L.) are cultivated for its seeds, the dry seeds are used in cooked beans, amanatto, and bean paste in Japan. Beans must be cooked or processed before consumption. Cooking brings about a number of changes in the physical characteristics and chemical composition of food legumes (Xu et al. 2011).

In the present study, we investigated antioxidant activity and α-glucosidase inhibitory activity of polyphenols in red kidney beans (RKB) upon thermal processing.

Materials and Methods

1. Materials

Samples of kidney beans were purchased from the Kawanishi Agricultural Cooperative Association (Obihiro, Japan). They were a dark red bean called red kidney bean (RKB), and a white bean called white kidney bean (WKB). 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. Thermal Processing

30g of kidney beans were added with 150 mL of distilled water, and soaked for 12 h at 20°C. The soaked liquid and beans were cooked with pressure cooker (Panasonic Co., Ltd., Osaka, Japan) at 113°C for 5 min. Cooked beans and cooking liquid were separated and stored at -20°C in dark place.

3. Extract Preparation and Fractionation

Unprocessed kidney beans were ground into a powder, and cooked beans were homogenized using a Teflon homogenizer, and added with 20 mL of methanol-HCl (99:1 v/v) and treated with ultrasound for 30 min, respectively. The mixture was centrifuged at 1,006 × g for 10 min to obtain a supernatant. The same extraction process was repeated two more times. Then, the residues were extracted three times with 80% ethanol, followed by three times with 70% acetone-water (20 mL solvent, 30 min of ultrasonication, followed by centrifugation each time). 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. Cooking liquid was directly through Diaion HP-20 columns, and fractionated by Sephadex LH-20 column chromatography.

4. Quantification of Polyphenols

Polyphenols were quantified using the Folin–Ciocalteu method (Miyashita et al. 2007). 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% Na_2CO_3 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 100 gram of beans (mg/100 g).

5. Quantification of Anthocyanin

The amount of anthocyanin was estimated according to the method described by Sellappan et al. (2002). Two 0.2 mL aliquots of the methanol fraction (HP-20 column) were separately mixed with 1.8 mL of 0.025 M potassium chloride buffer (pH 1.0) and 0.4 M sodium acetate buffer (pH 4.5). The absorbance of the mixtures was measured at 510 and 700 nm. The difference in absorbance between the two pH values and wavelengths was used to calculate the anthocyanin content as cyanidin-3-glucoside with molecular weight of 449.2 g/mol and molar absorptivity of 26,900 L/cm/mol. The anthocyanin content was expressed in mg per 100 gram of beans (mg/100 g).

6. Estimation of DPPH Radical Scavenging Activity

DPPH radical scavenging activity was evaluated by the method described by Brand-Williams et al. (1995), 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 mmol trolox equivalents per 100 gram of beans (mmol/100 g).

7. α-Glucosidase Inhibitory Activity

α-Glucosidase inhibition was analyzed following the methods of Matsumoto et al. (1993), 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 IC_{50} value.

8. 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 $p < 0.05$.

Results and Discussion

1. Polyphenols, Anthocyanin and DPPH Radical Scavenging Activity upon Thermal Processing

RKB were processed by pressure cooker, cooked beans (RKB-CB) and cooking liquid (RKB-CL) were obtained. The extraction was purified by HP-20 column, polyphenols, anthocyanin and DPPH radical scavenging activity were examined (Table 1). Polyphenols were 203.79 mg and 61.66 mg, anthocyanin content was 3.24 mg and 1.32 mg, and DPPH radical scavenging activity was 61.34 mmol and 20.73 mmol in RKB-CB and RKB-CL from 100 g RKB, respectively. RKB-CB showed higher polyphenols and DPPH radical scavenging activity than those of RKB-CL. However, DPPH radical scavenging activity (per mg polyphenol) was 3.01 μmol for RKB-CB, and 3.35 μmol for RKB-CL, the latter showed significantly higher DPPH radical scavenging activity. As compared to the unprocessed RKB, processed treatment (total values of cooked beans and cooking liquid) caused decreased in polyphenols (34%), anthocyanin content (76%), and DPPH radical scavenging activity (32%). These losses might be attributed to the breakdown of polyphenols during processing. Xu et al. (2011) reported that the polyphenols was decreased by thermal treatment in pinto beans, black beans and black soybeans, however, was increased in yellow soybeans.

Moreover, we analyzed polyphenols, anthocyanin and DPPH radical scavenging activity for WKB upon thermal processing. Polyphenols in RKB (400.39 mg/100 g seed) were higher than those of WKB (54.32 mg/100 g seed). White kidney beans' cooked beans (WKB-CB) showed higher polyphenols and DPPH radical scavenging activity than those of white kidney beans' cooking liquid (WKB-CL), and lower than RKB-CB and RKB-CL. Anthocyanin was not detected in WKB, WKB-CB, and WKB-CL. We found positive correlation (correlation coefficient, 0.98) between polyphenols and DPPH radical scavenging activity in unprocessed beans, cooked beans and cooking liquid of kidney beans. A positive correlation between the polyphenols and DPPH radical scavenging activity for common beans has been reported by Ci et al. (2017). Moreover, we examined polyphenols and DPPH radical scavenging activity for seed coat and cotyledon from RKB and WKB. Seed coat from RKB showed the highest polyphenols and DPPH radical scavenging activity, and seed coat of WKB showed the lowest polyphenols and DPPH radical scavenging activity. Cotyledon from RKB showed the higher polyphenols and DPPH radical scavenging activity than those of cotyledon from WKB. Shahidi et al. (2001) and Troszynska et al. (1997) reported the seed coat acts as a protective barrier for the cotyledon, has a high concentration of phenolic compounds. The same results were shown for RKB, in contrast, seed coat shown lower polyphenols than those of cotyledon for WKB. So thus polyphenol content may be related to the characteristic of polyphenol distribution for seed coat and cotyledon.

Table 1. Polyphenols, anthocyanin, and DPPH radical scavenging activity in RKB upon thermal processing.

	Polyphenols (mg/100 g seed)	Anthocyanin (mg/100 g seed)	DPPH radical scavenging activity (mmol/100 g seed)	DPPH/PP (μmol/mg)
RKB	$400.39^{a} \pm 2.00$	$17.43^{a} \pm 0.10$	$119.87^{a} \pm 1.62$	$3.00^{b} \pm 0.03$
RKB-CB	$203.79^{b} \pm 0.80$	$3.24^{b} \pm 0.20$	$61.34^{b} \pm 0.34$	$3.01^{b} \pm 0.02$
RKB-CL	$61.66^{c} \pm 2.40$	$1.32^{c} \pm 0.01$	$20.73^{c} \pm 0.24$	$3.35^{a} \pm 0.10$

Abbreviations: RKB, red kidney beans; RKB-CB, red kidney beans' cooked beans; RKB-CL, red kidney beans' cooking liquid. Values represent mean ± S.E.M. Differences between values superscripted with different letters were significant ($p < 0.05$).

2. Polyphenol Fractions

We performed Sephadex LH-20 column chromatography to obtain three polyphenol fractions, i.e., Fraction I (Fra. I), Fraction II (Fra.II), and Fraction III (Fra.III), for RKB upon thermal processing. According to Saito et al. (2007), Fra.I contains monomeric polyphenols, Fra.II contains oligomeric polyphenols, and Fra.III contains polymeric polyphenols. RKB and RKB-CL showed the similar profiles, monomeric polyphenols (Fra.I) represented 33% and 34%, oligomeric polyphenols (Fra.II) represented 67% and 66%, and polymeric polyphenols (Fra.III) was not detected, respectively (Figure 1). However, RKB-CB had mostly monomeric polyphenols (Fra.I, 83%) with small amounts of oligomeric polyphenols (Fra.II, 13%) and polymeric polyphenols (Fra.III, 4%). Monomeric polyphenols were mainly in WKB, WKB-CB, and WKB-CL, respectively.

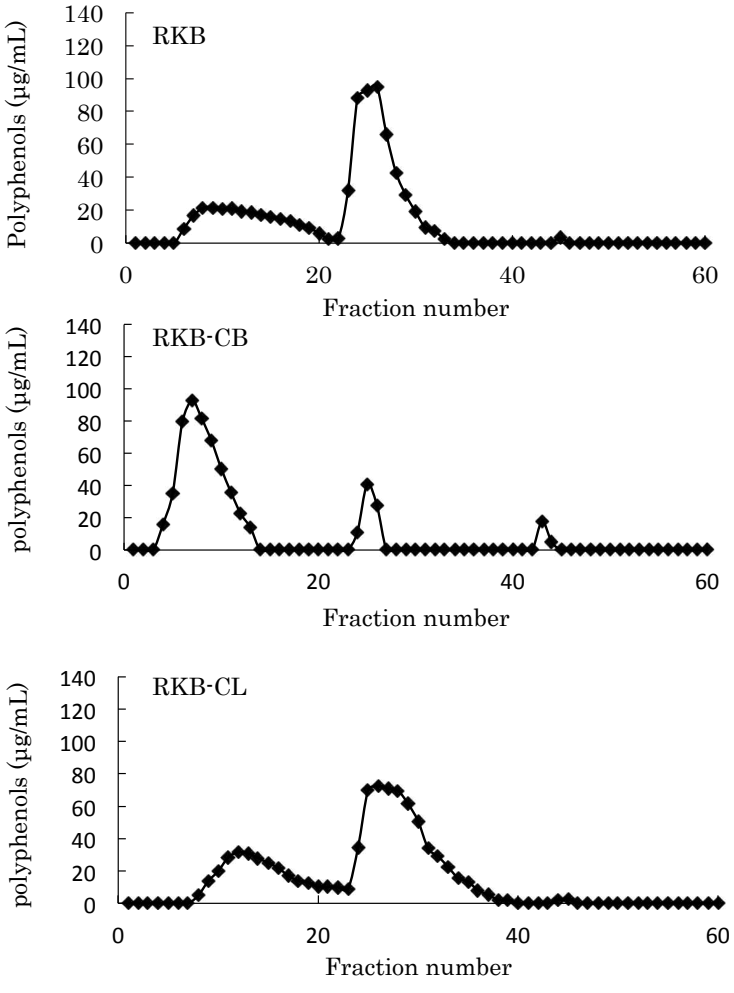

Figure 1. Sephadex LH-20 column chromatogram of polyphenols prepared in RKB upon thermal processing. The polyphenol fraction obtained by column chromatography on Diaion HP-20 was applied to column chromatography on Sephadex LH-20 and eluted with ethanol, fraction number 1-20 (Fra.I); methanol, fraction number 21–40, (Fra.II); and 60% acetone, fraction number 41–60, (Fra.III). The abbreviations are the same as Table 1.

3. α-Glucosidase Inhibitory Activity upon Thermal Processing

We analyzed the inhibitory activities of polyphenols on α-glucosidase in RKB upon thermal processing (Table 2). Polyphenols from RKB, RKB-CB and RKB-CL inhibited the activity of α-glucosidase. Phenolic compounds from scarlet runner beans (Ci et al. 2018), soybeans (Ademiluyia et al. 2013), the millet seed coat (Shobana et al. 2009), and seven kinds of legume (Saito et al. 2007) inhibit α-glucosidase activity. RKB-CL showed the highest α-glucosidase inhibitory activity (IC_{50} value, 4.0 μg/mL), followed by RKB (IC_{50} value, 6.0 μg/mL) and RKB-CB (>80.0 μg/mL). On α-glucosidase inhibitory activity, 10 μg/mL polyphenols for Fra.I was 6.05%, 5.32%, and 10.24% in RKB, RKB-CB and RKB-CL, respectively (Figure 2). Comparing to Fra. I, Fra.II showed higher α-glucosidase inhibitory activity in RKB (70.43%), RKB-CB (8.32%) and RKB-CL (61.40%), respectively. In our daily cooking and cooking beans factory, the cooking liquid was thrown away. In our research, RKB-CL showed higher α-glucosidase inhibitory activity. RKB-CL is worth of reusing, and developing as nutraceuticals.

Table 2. The inhibitory concentration of 50% of α-glucosidase activity (IC_{50}) in RKB upon thermal processing.

	IC_{50} (μg/mL)
RKB	4.0
RKB-CB	> 80.0
RKB-CL	6.0

The abbreviations are the same as Table 1.

Conclusions

Polyphenols from RKB-CL showed higher DPPH radical scavenging activity (per mg polyphenol) and α-glucosidase inhibitory activity than those of RKB-CB. RKB-CB had mostly monomeric polyphenols, and RKB-CL had high ratio of oligomer polyphenols. Moreover, we found oligomeric polyphenols from RKB-CL was related to inhibiting α-glucosidase activity. These observations indicate that RKB-CL may serve as a source for the development of nutraceuticals with anti-diabetic and antioxidant activity.

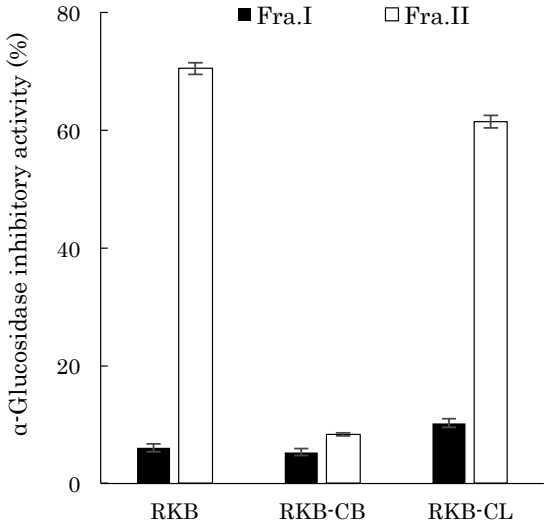

Figure 2. α-Glucosidase inhibitory activity of Fra.I and Fra.II in RKB upon thermal processing. Polyphenols of each Fra.I and Fra.II was used at 10 μg/mL. The abbreviations are the same as Table 1.

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摘　要

赤金時豆 100 ｇから調製した煮豆に含まれる HP カラムで精製したポリフェノール含量は 203.79 ㎎、煮汁に含まれるその含量は 61.66 ㎎であった。赤金時豆煮豆の HP カラムで精製したポリフェノールは、主にモノマー型が多く含まれていたが、煮汁のそれにはオリゴマー型が多く含まれていた。また、赤金時豆 100 ｇから調製した煮豆に含まれるポリフェノールの DPPH ラジカル消去活性は 61.34 ㎜ol、煮汁のそれは 20.73 ㎜ol で、前者の方が有意に高い値を示した。また、赤金時豆煮汁の HP カラムで精製したポリフェノールには高いα-グルコシダーゼ活性阻害作用（IC_{50} 値，6.0 μg/mL）が認められたが、煮豆ポリフェノールには極弱い阻害作用（IC_{50} 値，＞80 μg/mL）しか認められなかった。これらの結果は、赤金時豆の煮豆のポリフェノールと抗酸化活性は煮汁のそれらよりも高いこと、及び煮豆ポリフェノールのα-グルコシダーゼ活性阻害作用は煮汁ポリフェノールのそれに比べて弱いことを示している。また、煮汁ポリフェノールの LH カラムで得られたメタノール画分（Fra. II）に主にα-グルコシダーゼ阻害活性が認められ、オリゴマー型ポリフェノールが活性阻害に関係していることが考えられる。

キーワード：金時豆、ポリフェノール、DPPH ラジカル消去活性、α-グルコシダーゼ活性阻害