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Characterisation of anthocyanins and proanthocyanidins of adzuki bean extracts and their antioxidant activity

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ABSTRACT

A novel extract powder purified from the boiled water of adzuki bean paste production was developed to better utilize this resource. The compounds contributing to pigmentation of purified adzuki bean extract powders were investigated in order to compare their antioxidant activity *in vitro* with (+)-catechin. When a normal extract was exposed to the air under heat treatment, the colour of adzuki bean extract became more strongly reddish, which was associated with polyphenol polymerization. Anthocyanins also contributed to the pigmentation of the purified adzuki bean extracts. Especially, two anthocyanin compounds, peonidin-3-rutinoside and malvidin-3-O-glucoside were newly identified in the adzuki bean extract. The reducing powder, iron chelating activity and free-radical scavenging capacity of the adzuki bean extract were greater than that of (+)-catechin while its total antioxidant value was lower. Thus, adzuki bean extract powders are promising alternatives to replace synthetic antioxidants and potential dyes.

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1. Introduction

Legumes are a staple food in many countries and an excellent source of natural bioactive compounds. Among them, red adzuki bean (*Vigna angularis*) is cultivated throughout East Asia, where it is traditionally used for making bean pastes for use in confectioneries. As high polyphenol content in kidney bean (*Phaseolus vulgaris* L.) seed coats have been observed (Chen et al.,

2014), the adzuki bean seed coat is also high in polyphenols (Lin & Lai, 2006); these polyphenols have been dominantly identified to contain catechin glycosides, quercetin glycosides, myricetin 3-rhamnoside, anthocyanin, and procyanidin dimers (Amarowicz, Estrella, Hernandez, & Troszynaska, 2008; Ariga, Koshiyama, & Fukushima, 1988). The bioactive compounds in the adzuki bean seed coat have received significant interest because of their health-promoting antioxidant properties (Lin & Lai, 2006). Aroma extracts from adzuki bean seed coat are

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Chemical compounds: Peonidin-3-rutinoside (CID 44256842); Pelargonidin-3-O-glucoside (CID 443648); Malvidin-3-O-glucoside (CID 443652); (+)-catechin (CID 9064).

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reported to inhibit the formation of malonaldehyde (Lee, Mitchell, & Shibamoto, 2000), which is a marker for oxidative stress. A decrease in vascular oxidative stress and inflammation has also been reported in rats fed with the polyphenol-containing adzuki bean seed coat (Mukai & Sato, 2011). Furthermore, Kitano-Okada et al. (2012) have shown *in vitro* that extracts from adzuki bean seed coat inhibit the activity of pancreatic lipase. These inhibitions would explain the results of a clinical trial that suggests that the consumption of the adzuki bean is linked to a reduced risk of lifestyle-related diseases in humans (Maruyama et al., 2008).

To manufacture the adzuki bean paste, the beans are boiled in water, which is then generally discarded after completing the boiling process. However, the pigments contained in this water are receiving increased interest because of the need to maximize the utilization of natural resources in order to decrease the associated carbon footprint. Moreover, the food industry is experiencing an increasing demand for natural alternatives to replace synthetic food additives (e.g., antioxidants) (Amarowicz, Naczek, & Shahidi, 2000). In addition, adzuki bean extract is a potential source of pigments (food colourants) because the water used to boil the beans becomes strongly red or purple. Therefore, a novel extract powder purified from the water of adzuki bean paste production was developed and introduced in Japan to better utilize the resource (Adzuki-nomoto, Cosmo Foods Co., Ltd, Tokyo, Japan). The extract powder is highly purified and rich in natural polyphenols (Kitano-Okada et al., 2012). Interestingly, hot air-exposed purified adzuki bean extract rather maintains consistent colour strength than the normal purified adzuki bean extract because polyphenols in the adzuki bean extract were polymerized by oxidation, and both the extracts are stable to light, heat, and pH changes. However, information on responsible compounds for pigmentation as well as the potent antioxidative activity of the different purified adzuki bean extract powders is very limited.

Therefore, the objective of this investigation was to compare the different purified adzuki bean extract powders from industrial residue in order to reveal their bioactive compounds for pigmentation and *in vitro* functional attributes such as reducing power and radical scavenging activity assay. For this, the antioxidant activity was compared with (+)-catechin as standard polyphenol.

2. Materials and methods

2.1. Reagents, chemicals, and standards

Calibrations were performed by using standard compounds (catechin, cyanidin-3-O-glucoside, pelargonidin-3-O-glucoside, peonidin-3-O-glucoside, and malvidin-3-O-glucoside) from Sigma-Aldrich (St Louis, MO, USA). Solvents (ethanol, butanol, acetonitrile and formic acid) and concentrated hydrochloric acid (HCl) were purchased from Wako Chemical Co., Ltd. (Tokyo, Japan). Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), malondialdehyde, 2,2-azobis(2-methylpropionamide) dihydrochloride (AAPH), 2,2-diphenyl-1-picrylhydrazyl (DPPH), thiobarbituric acid, and Folin–Ciocalteu phenol reagent were obtained from Sigma-Aldrich. Ferric ammonium sulphate and ferrozine were purchased from Kishida Chemical Co., Ltd.

(Osaka, Japan). All other chemicals were purchased from Kanto Chemical Co., Ltd. (Tokyo, Japan).

2.2. Adzuki bean extract powder (AEP)

The adzuki bean extract powder (AEP; also known as Adzuki-nomoto) made from the simmering water obtained during the sweetened adzuki bean paste production was kindly supplied by Cosmo Foods. The extract powder was made by the following procedure: first the bean was boiled and cooled. The water was collected from supernatant, adjusted at pH 4.0, treated with 0.005% pectinase HL (Yakult Pharmaceutical Industry Co., Ltd, Tokyo, Japan) and passed through a 50 mesh sieve to separate undigested materials. It was further applied to an ultrafiltration device to remove polymeric components and adjusted at pH 8.5. Then two different types of adzuki bean extract powder were made depending on the process with and without oxidative polymerization of polyphenols. One was sterilized (115 °C for 90 min) and spray-dried (AEP-1). The other was exposed to the air under heat treatment (90 °C for 5 h) and spray-dried (AEP-2).

2.3. Total polyphenols and proanthocyanidins analyses

The total concentration of polyphenols in the AEP samples was determined according to the Folin–Ciocalteu's method using (+)-catechin as a standard (Singleton, Orthofer, & Lamuela-Raventos, 1999). The absorbance was read at 750 nm using a spectrophotometer (1600-UV; Shimadzu, Kyoto, Japan). The results were expressed in mg of (+)-catechin equivalents to per gram of bean extract powder. All tests were performed in triplicate.

For assay of total proanthocyanidins concentration in the AEP samples, the acid butanol method was employed (Porter, Hrstich, & Chan, 1986). In brief, 6.0 mL of the butanol-HCl reagent (95:5, v/v) were added to 1.0 mL of suitably diluted extract (1 mg/mL) in a screw cap test tube. Then 0.2 mL of ferric reagent (2% ferric ammonium sulphate in 2M HCl) was added to the solution. The container was capped, mixed and boiled for 30 min in a water bath. Similarly, a standard curve was prepared using procyanidin B-2 (Sigma-Aldrich) ranging from 0 to 0.25 mg/mL. After cooling, the absorbance was read at 550 nm using a spectrophotometer. The results were expressed in mg of procyanidin B-2 equivalents to per gram of bean extract powder.

For quantification of oligomeric proanthocyanidins, the AEP sample was dissolved in water (1 mg/mL) and filtered using a 0.45 µm polytetrafluoroethylene (PTFE) hydrophilic syringe filter (Advantec Dismic-13HP, Toyo Roshi Co., Ltd., Tokyo, Japan). The filtrate was loaded into a Shimadzu Prominence HPLC system equipped with an LC-20AD pump (Shimadzu). The analytes were separated using a TSKgel ODS-80Ts column (4.6 mm × 250 mm, Tosoh, Tokyo, Japan) with photodiode array detector (SPD-M20A, Shimadzu). Absorbance was monitored at 210–600 nm. The column oven temperature was set at 40 °C. The injection volume was 10 µL and the flow rate was 1.0 mL/min. The mobile phases were made of 0.05% trifluoroacetic acid (TFA) in water (v/v, mobile phase A) and 0.05% TFA in 90% acetonitrile (v/v, mobile phase B). Elution was used as a linear gradient from 5 to 35% B in 13 min, from 35 to 70% B in 20 min, and then 70%

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