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Research Article

Quality Characteristics and Antioxidant Activities of Rice/Adzuki Bean Mixtures Cooked Using Two Different Methods

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This study explored the optimal preparation and the antioxidant levels of rice/adzuki bean mixtures. We compared the quality and physicochemical characteristics of cooked mixtures of rice and adzuki beans prepared using normal and high-pressure rice cookers, with and without the addition of alcohol (15%, v/v). The water-binding capacity and swelling power decreased upon addition of adzuki beans, but water solubility increased. The peak, trough, final, and setback viscosities decreased, but the breakdown viscosity increased. The total polyphenol and flavonoid contents increased after addition of adzuki beans. Total polyphenol contents in cooked rice/20% (w/w) Arari and Geomguseul bean mixtures in a normal cooker with addition of alcohol were 3.00 and 3.09 times higher than plain rice. The flavonoid contents were 10.33 and 8.90 times higher than plain rice. The predominant phenolic acids in cooked rice/Arari bean mixtures were *p*-coumaric acid and *trans*-3-hydroxycinnamic acid, and in cooked rice/Geomguseul bean mixtures, they were syringic acid and *trans*-3-hydroxycinnamic acid. Overall, phenolic acid levels were higher in mixtures cooked in plain water. The DPPH- and ABTS-radical-scavenging activities increased upon addition of adzuki beans. DPPH radical-scavenging activities in cooked rice/20% (w/w) Arari and Geomguseul bean mixtures in a normal cooker with addition of alcohol were 9.09 and 9.22 times higher than plain rice. ABTS radical-scavenging activities were 8.74 and 9.01 times higher than plain rice. Moreover, rice/adzuki bean mixtures prepared in a normal cooker, with addition of alcohol, exhibited higher antioxidant levels than other samples. We present the antioxidative properties of rice/adzuki bean mixtures prepared in different ways; these data will aid manufacturers.

1. Introduction

Adzuki beans (*Vigna angularis*) are cultivated and consumed principally in East Asia [1], where the adzuki beans are traditionally used to make confectionery pastes [2]. Compared with other commercial starches, adzuki bean starch affords certain advantages, including noninduction of chronic disease because of the relatively low insulin response, a high paste shear resistance, and good granule stability [3]. The adzuki beans can be used to prepare various foods, including adzuki bean pastes, desserts, pastries, and drinks, and can improve taste, flavor, and viscosity. The growing demand for starch has created a strong interest in novel starch sources [4].

Recently, many studies have reported that adzuki bean extracts exert a variety of physiological functions, including antioxidant, anti-inflammatory, antiatherosclerosis, anticancer, and procardiovascular activities [5, 6]. An adzuki bean extract suppressed obesity [7], reduced blood pressure [8], reduced the serum cholesterol level [9], suppressed hyperglycemia [10], and reduced the numbers of infiltrating macrophages and attenuated glomerular expansion in mouse and rat models of streptozoicin-induced diabetic nephropathy [11]. Flavonoids in the adzuki bean may mediate such beneficial effects [8, 9, 11].

Oxidative stress is characterized by excessive production of reactive oxygen species (ROS) combined with an inadequate or defective antioxidant defense system [12].

Oxidative stress greatly affects various biological structures, including membranes, lipids, proteins, and nucleic acids [13]. Therefore, the antioxidant activities of various grains have been intensively studied. Also, processing industries seek to minimize the loss of nutritionally valuable components, not create harmful compounds, be ecofriendly, and be economically viable. Minimally processed foods are thus of increasing importance [14]; such foods afford greater health benefits [15].

Here, we explored the optimal preparation (with and without alcohol) [16] and the antioxidant levels of rice/adzuki bean mixtures. We sought to optimize both eating quality and functional value.

2. Materials and Methods

2.1. Sample Preparation. We used adzuki beans of the cultivars *Vigna angularis* var. Nipponensis cv. Arari and cv. Geomguseul, and rice of the cultivar *Oryza sativa* cv. Samkwang. The adzuki bean cultivars were grown at the National Institute of Crop Science, Rural Development Administration, Miryang, South Korea, during the 2015 growing season. White rice was prepared using a rice huller (model SY88-TH, Ssangyong Ltd., Incheon, Korea) and a milling machine (model MC-90A, Satake, Hiroshima, Japan). All samples were stored in a refrigerator at 4°C. The raw materials were pulverized using a microhammer/cutter mill (Type 3; Culatti AG, Zürich, Switzerland) to allow for qualitative analysis.

2.2. Analysis of Pasting Characteristics. The pasting characteristics of rice prepared with different proportions of adzuki beans (adzuki beans at 0, 5, 10, 15, and 20% w/w) were measured using the methods of Kim et al. [17] and a rapid viscosity analyzer (Model RVA-3D; Newport Scientific, Warriewood, NSW, Australia). Rice/adzuki bean mixtures were pulverized to ≥60-mesh (3 g samples). Each sample was placed in an aluminum can; dispersed in 25 mL distilled water; held at 50°C for 1 min and then raised to 95°C over 3.48 min; maintained at 95°C for 2.05 min; and cooled to 50°C over 3.48 min, and the viscosity characteristics were assessed. The total experimental time was about 13 min. The peak, trough, breakdown, final, and setback viscosities were measured.

2.3. Water-Binding Capacity, Swelling Power, and Water Solubility. The water-binding capacities of various rice/adzuki bean mixtures (adzuki beans at 0, 5, 10, 15, and 20% w/w) were measured by mixing 1 g of pulverized samples with 40 mL distilled water followed by stirring for 1 h [18]. Supernatants were removed by centrifugation at 1,500 ×g for 10 min, and the weights of precipitated powders were measured. Each water-binding capacity was calculated by subtracting the initial sample weight from the weight of the precipitated sample and is presented as a percentage of the initial sample weight. Swelling power and water solubility were measured by dispersing 1 g of pulverized samples in 30 mL distilled water and heating at 90°C (±1) for 30 min followed by centrifugation at 1,500 ×g for 20 min. The supernatants were dried at 105°C for 12 h and weighed, and the precipitates were also weighed [19].

2.4. Cooking Methods. Rice/adzuki bean mixtures were prepared with 0, 5, 10, 15, and 20% w/w adzuki beans, washed three times, soaked in water at 25°C for 30 min, and drained. Water with or without alcohol (for cooking; 120 mL water or 100 mL water with 20 mL alcohol (based on data from a preliminary study)) was then added. Cereal functionality improved upon addition of alcohol to a rice/barley mix [16]. A normal rice cooker (Cuckoo CR-0671V, Seoul, Korea) and a high-pressure cooker (Cuckoo EHS035FW) were used. Samples were boiled or steamed for 15 min.

2.5. Measurement of Phenolic Compounds. The levels of phenolics measured in, and the radical-scavenging activity of, the various cooked samples, which were homogenized in 80% (v/v) ethanol (homogenizer model: HG-15A; Daihan Scientific Co., Ltd., Seoul, Korea); shaken at room temperature (25°C) for 24 h (WiseCube WIS-RL010, Daihan Scientific Co., Ltd.); filtered (Advantec, Toyo Roshi Kaisha, Ltd., Tokyo, Japan); and stored at −20°C. Total polyphenol levels were measured using the Folin–Ciocalteu method [20]. Standards or extracts (10 μL) were mixed with 200 μL of a sodium carbonate solution (2% w/v) and 10 μL of the Folin–Ciocalteu reagent (Sigma-Aldrich, St. Louis, MO, USA; 50% v/v). The mixtures were incubated for 30 min at room temperature, and absorptions at 750 nm were measured. The results are expressed as μg gallic acid (Sigma-Aldrich) equivalents per g of sample. To measure total flavonoid levels, standards or extracts (50 μL) were mixed with 200 μL water and 15 μL $NaNO_2$ (5%, w/v). After 5 min, 30 μL of $AlCl_3{\cdot}6H_2O$ (10%, w/v) was added, and incubation proceeded for another 6 min. Reactions were terminated by addition of 1 M NaOH (100 μL), and absorbances at 510 nm were measured. The results are expressed as μg catechin (Sigma-Aldrich) equivalents per g of sample. All extracts were analyzed in triplicate.

2.6. Measurement of Individual Phenolic Acid Levels. The phenolic acid composition of each extract was determined via HPLC as described by Kim et al. [21]. The column was employed an ODS column (5 μm, 4.6 × 250 mm; Agilent Technologies, Santa Clara, CA, USA). Gradient elution featured solvent A (water with 0.1% (v/v) acetic acid) and solvent B (acetonitrile with 0.1% (v/v) acetic acid). The gradient program was as follows: 0–2 min, 92–90% A in B (gradient); 2–27 min, 90–70% A in B (gradient); 27–50 min, 70–10% A in B (gradient); 50–51 min, 10–0% A in B (gradient); 51–60 min, 0% A in B (isocratic); and 60–70 min, 0–92% A in B (gradient). The flow rate was 1 mL/min, and the injection volume 20 μL. The UV detector was set to 280 nm. A phenolic acid standard mixture containing 4-hydroxybenzoic acid, vanillic acid, rutin, protocatechuic acid, myricetin, quercertin, kaempferol, gallic acid, syringic

acid, *trans*-3-hydroxycinnamic acid, 2-hydroxy cinnamic acid, naringin, cinnamic acid, naringenin, chlorogenic acid, caffeic acid, *p*-coumaric acid, ferulic acid, sinapinic acid, and salicylic acid (Sigma-Aldrich) was prepared in HPLC-grade methanol. The phenolic acid concentrations were determined by reference to standard curves obtained by injecting different concentrations of standards into the HPLC system. Peaks were verified by addition of standards to the samples, and all peak areas were calculated by reference to those of standard peaks. Total phenolic acid contents were calculated by summing the levels of individual phenolics.

2.7. Measurement of DPPH- and ABTS-Radical-Scavenging Activities. DPPH- and ABTS-radical-scavenging activities were measured according to [20], with some modifications. An 800 μL aliquot of a 0.2 mM DPPH (1,1-diphenyl-2-picrylhydrazyl, Sigma-Aldrich) methanolic solution was mixed with 200 μL of each sample, shaken vigorously, left to stand for 30 min under low light, and absorbance at 515 nm was measured. The ABTS cationic radical was generated by adding ABTS (2,2′-azino-bis-3-ethylbenzo-thiazoline-6-sulfonic acid, Sigma-Aldrich) to 7 mM to a 2.45 mM potassium persulfate solution followed by holding the mixture overnight in the dark at room temperature. The radical solution was diluted with methanol to an absorbance of 1.4–1.5 at 735 nm (molar extinction coefficient, $\varepsilon = 3.6 \times 10^4\ \text{mol}^{-1}\cdot\text{cm}^{-1}$). The diluted ABTS radical solution (1 mL) was added to 50 mL of each extract, a Trolox standard solution, or distilled water. After 30 min, absorbances were spectrophotometrically measured at 735 nm (Multiskan™ GO Microplate spectrophotometer, Thermo Fisher Scientific, Waltham, MA, USA). Both scavenging activities were expressed as Trolox-equivalent antioxidant capacities (TEACs), thus mg TE/100 g of sample.

2.8. Statistical Analysis. All data are expressed as means ± standard deviations (SDs). Significant differences among treatments were determined by one-way analysis of variance (ANOVA) using Duncan's multiple range test, with the aid of SAS software ver. 9.2 (SAS Institute, Cary, NC, USA); the significance level was set to 0.05.

3. Results and Discussion

3.1. Pasting Characteristics. The pasting characteristics by adzuki bean were proportion determined; the peak, trough, breakdown, final, and setback viscosities were measured (Table 1). As the adzuki bean ratio increased, the pasting viscosities decreased, except for the breakdown viscosity (the difference between the peak and trough viscosities). Amylose content correlated negatively with viscosity but highly with the heat and shear resistances evident during processing [22]. The breakdown viscosity of mixtures containing both Arari and Geomguseul beans (5–20% w/w) were 73.2–76.8 and 71.4–79.0 RVU, respectively, and did not vary by the adzuki bean proportion. The final viscosity was measured after completion of both heating and cooling; starch particles (such as amylose) then recombine to increase viscosity [22]. As the adzuki bean proportion increased, the final viscosities of mixtures containing both Arari and Geomguseul beans fell to 138.6–195.8 and 134.5–195.5 RVU, respectively. The setback viscosity reflects starch aging; the higher the value, the more rapid the aging [22]. The setback viscosity is obtained by subtracting the peak from the final viscosity; the higher this value, the faster the aging; lower values indicate slower aging and a longer duration of the desired taste. The inverse relationship between the proportion of adzuki beans and setback viscosity was found. Thus, the higher the adzuki bean proportion, the slower the aging.

3.2. Water Characteristics. Table 2 shows that water-binding decreased with increasing adzuki bean proportions (0, 5, 10, 15, 20, and 100% w/w). The water-binding capacities of mixtures of rice with Arari and Geomguseul beans (0–20% w/w) were 109.9–125.7 and 119.4–147.9%, respectively. Water-binding capacity is an indicator of the affinity of the sample for water and increases as the amorphous proportion of starch increases, related to the swelling index [23]. The water-binding force indicates the extent of water-binding to powdered grains, reflecting water penetration into amorphous regions of starch particles or water absorption onto particle surfaces [24]. Following the addition of Arari and Geomguseul beans (0–20% w/w) to rice, water solubility increased significantly from 4.3 to 8.7% and 5.3 to 10.2%, respectively, and swelling power decreased significantly from 207.2 to 93.6% and 169.2 to 76.1%, respectively, as the adzuki bean proportions increased. The swelling power and water solubility index measure the interactions between amorphous starch chains and the crystalline domains of starch particles and are affected by the proportions of amylose-lipid complexes, amylose, and amylopectin [17]. A reduction in swelling power was thought to be attributable to differences in the compositions of various barley components, which have relatively higher lipid but lower sugar contents than white rice [25]. An increase in solubility is thought to be attributable to elongation of amylose chains or increased soluble carbohydrate levels when barley is swollen by heat, and lipids and fibers collapse [26].

3.3. Phenolic Compounds of Rice Cooked with Different Proportions of Adzuki Beans. Phenolics are the major antioxidants of fruits, vegetables, and grains [27]. Therefore, we measured polyphenolic levels and their antioxidant contributions. The polyphenolic levels of rice/adzuki bean mixtures with different adzuki bean proportions are shown, by cooking method, in Table 3. The total polyphenol content of plain rice varied by the cooking method from 119.5–120.6 μg GAE/g. The total polyphenol contents of mixes with 20% (w/w) Arari beans cooked in water averaged 351.4 ± 3.2 and 325.7 ± 1.5 μg GAE/g (normal and high-pressure cooking, resp.); the figures for the same mixtures cooked with 10% (v/v) alcohol were 361.4 ± 0.9 and 346.3 ± 1.8 μg GAE/g (Table 3). The figures for rice/adzuki bean mixtures with 5–20% (w/w) Geomguseul beans were

Table 1: Pasting characteristics with different mixing ratio of adzuki bean.

Variety	Mixing ratio of adzuki bean (%)	Peak viscosity (RVU[1])	Trough viscosity (RVU)	Break down (RVU)	Final viscosity (RVU)	Setback (RVU)
Arari	0	195.0 ± 1.4[a2]	127.9 ± 2.5[a]	67.1 ± 3.9[b]	252.0 ± 2.3[a]	57.0 ± 3.6[a]
	5	193.8 ± 1.3[a]	118.2 ± 0.2[b]	75.6 ± 1.5[a]	195.8 ± 1.3[b]	2.0 ± 0.3[c]
	10	173.2 ± 0.6[b]	96.4 ± 1.2[c]	76.8 ± 1.0[a]	169.9 ± 1.1[c]	−3.4 ± 0.5[d]
	15	158.0 ± 0.3[c]	83.9 ± 0.8[d]	74.1 ± 1.0[a]	153.6 ± 1.4[d]	−4.4 ± 1.5[de]
	20	146.4 ± 0.8[d]	73.2 ± 2.1[e]	73.2 ± 2.2[a]	138.6 ± 1.1[e]	−7.8 ± 1.8[e]
Geomguseul	0	195.0 ± 1.4[a]	127.9 ± 2.5[a]	67.1 ± 3.9[c]	252.0 ± 2.3[a]	57.0 ± 3.6[a]
	5	192.8 ± 1.3[b]	116.4 ± 1.7[b]	76.4 ± 2.9[a]	195.5 ± 0.6[b]	2.7 ± 1.8[c]
	10	172.2 ± 2.0[c]	93.3 ± 0.6[c]	79.0 ± 2.6[a]	167.6 ± 0.2[c]	−4.7 ± 2.1[d]
	15	158.2 ± 0.8[d]	80.9 ± 0.7[d]	77.3 ± 1.5[a]	151.5 ± 0.7[d]	−6.8 ± 1.3[d]
	20	141.2 ± 0.8[e]	69.8 ± 0.9[e]	71.4 ± 1.6[b]	134.5 ± 1.7[e]	−6.7 ± 1.7[d]

[1]Rapid visco units; [2]all values are means ± SDs of those of triplicate determinations. Means bearing different superscripts within a column (a–f) differed significantly at $p < 0.05$ by one-way analysis of variance (ANOVA) using Duncan's multiple range test.

Table 2: Water-binding capacities, water solubility, and swelling power with different mixing ratios of adzuki bean.

Variety	Mixing ratio of adzuki bean (%)	Water-binding capacity (%)	Water solubility (%)	Swelling power (%)
Arari	0	122.8 ± 4.6[a1]	4.3 ± 0.1[f]	207.2 ± 4.4[a]
	5	109.9 ± 1.1[b]	6.2 ± 0.2[e]	144.9 ± 5.2[b]
	10	113.4 ± 0.5[b]	7.3 ± 0.4[d]	118.1 ± 7.4[c]
	15	115.2 ± 4.7[b]	8.0 ± 0.1[c]	104.8 ± 2.1[d]
	20	125.7 ± 1.5[a]	8.7 ± 0.1[b]	93.6 ± 0.9[e]
Geomguseul	0	123.5 ± 2.8[c]	5.3 ± 0.2[f]	169.2 ± 7.2[a]
	5	119.4 ± 1.1[c]	6.9 ± 0.2[e]	118.7 ± 4.9[b]
	10	122.6 ± 1.9[c]	7.7 ± 0.1[d]	103.5 ± 2.6[c]
	15	130.4 ± 4.1[b]	8.7 ± 0.2[c]	91.5 ± 3.2[d]
	20	147.9 ± 1.7[a]	10.2 ± 0.2[b]	76.1 ± 1.6[e]

[1]All values are the means ± SDs of those of triplicate determinations. Means bearing different superscripts within a column (a–f) differed significantly at $p < 0.05$ by one-way analysis of variance (ANOVA) using Duncan's multiple range test.

Table 3: Total polyphenol and flavonoid contents of the ethanolic extracts with different mixing ratio of adzuki bean (*Vigna angularis* var. nipponensis cv. Arari and cv. Geomguseul) and cooking methods.

Variety	Mixing ratio of adzuki bean (%)	Total polyphenol contents (μg gallic acid equivalents/g sample)				Total flavonoid contents (μg catechin equivalents/g sample)			
		GRC[1] (water)	HPRC[2] (water)	GRC (10% alcohol)	HPRC (10% alcohol)	GRC (water)	HPRC (water)	GRC (10% alcohol)	HPRC (10% alcohol)
Arari	0	120.6 ± 0.5[e3]	119.5 ± 2.7[e]	120.5 ± 2.7[e]	120.4 ± 1.3[e]	15.8 ± 0.0[e]	15.5 ± 0.2[e]	15.7 ± 0.0[e]	15.7 ± 0.2[e]
	5	148.1 ± 1.5[d]	146.6 ± 5.0[d]	159.6 ± 1.6[d]	148.4 ± 2.6[d]	50.7 ± 1.9[d]	56.7 ± 1.0[d]	45.7 ± 2.8[d]	47.0 ± 1.0[d]
	10	218.8 ± 3.1[c]	203.5 ± 1.4[c]	231.9 ± 1.8[c]	214.2 ± 4.6[c]	91.8 ± 1.5[c]	99.7 ± 1.0[c]	84.2 ± 0.9[c]	91.1 ± 1.5[c]
	15	303.8 ± 1.8[b]	273.9 ± 3.7[b]	310.4 ± 3.3[b]	276.5 ± 2.3[b]	146.2 ± 4.0[b]	144.9 ± 5.2[b]	126.3 ± 2.7[b]	133.1 ± 4.6[b]
	20	351.4 ± 3.2[a]	325.7 ± 1.5[a]	361.4 ± 0.9[a]	346.3 ± 1.8[a]	173.0 ± 1.0[a]	171.4 ± 5.8[a]	162.6 ± 1.5[a]	178.5 ± 4.8[a]
Geomguseul	0	120.6 ± 0.5[e]	119.5 ± 2.7[e]	120.5 ± 2.7[e]	120.4 ± 1.3[e]	15.8 ± 0.0[e]	15.5 ± 0.2[e]	15.7 ± 0.0[e]	15.7 ± 0.2[e]
	5	144.7 ± 3.0[d]	138.5 ± 0.8[d]	158.6 ± 2.4[d]	150.5 ± 1.5[d]	39.8 ± 1.5[d]	48.7 ± 2.1[d]	35.3 ± 1.6[d]	39.9 ± 1.0[d]
	10	222.4 ± 0.9[c]	207.6 ± 5.2[c]	226.2 ± 3.3[c]	206.2 ± 1.5[c]	84.5 ± 2.0[c]	85.2 ± 1.6[c]	69.1 ± 0.9[c]	77.9 ± 1.5[c]
	15	287.4 ± 0.9[b]	266.4 ± 1.5[b]	297.4 ± 1.6[b]	267.1 ± 3.1[b]	114.9 ± 3.1[b]	120.0 ± 1.0[b]	106.4 ± 1.0[b]	109.9 ± 2.1[b]
	20	338.6 ± 5.5[a]	327.4 ± 4.3[a]	372.5 ± 1.6[a]	331.9 ± 3.1[a]	141.0 ± 2.6[a]	168.5 ± 2.8[a]	140.1 ± 1.0[a]	137.3 ± 2.5[a]

[1]General rice cooker; [2]high-pressure rice cooker. All values are expressed as the mean ± SD of triplicate determinations; [3]all values are expressed as the mean ± SD of triplicate determinations. Means with different superscripts within a column (a–f) are significantly different at $p < 0.05$ by one-way analysis of variance (ANOVA) using Duncan's multiple range test.

144.7–338.6 and 138.5–327.4 μg GAE/g (water; normal and high-pressure cooking), and 158.6–372.5 and 150.5–331.9 μg GAE/g (10% (v/v) alcohol; normal and high-pressure cooking), respectively (Table 3). Of all cereal antioxidants, polyphenolics are the most powerful, as the phenolic ring stabilizes free radicals [19].

The total flavonoid content of mixtures increased as the adzuki bean proportion increased (Table 3). The total flavonoid contents of plain rice were 15.7–15.8 and 15.5–15.7 μg CE/g depending on the cooking method (normal and high-pressure, resp.). The total flavonoid contents of rice with 20% Arari beans cooked in water were

Table 4: The phenolic acid contents of ethanolic extracts of cooked mixtures of rice with various proportions of adzuki beans (*Vigna angularis* var. nipponensis cv. Arari).

Mixing ratio of adzuki bean (%)		Only water								Added 10% fermented alcohol							
		Normal rice cooker				High-pressure rice cooker				Normal rice cooker				High-pressure rice cooker			
Phenolic compound ($\mu g\ g^{-1}$)	Raw material (Arari)	5	10	15	20	5	10	15	20	5	10	15	20	5	10	15	20
4-Hydroxybenzoic acid	5.98 ± 0.15	0.05 ± 0.04	0.54 ± 0.02	0.47 ± 0.02	0.80 ± 0.04	0.16 ± 0.05	0.38 ± 0.00	0.68 ± 0.04	1.09 ± 0.08	0.08 ± 0.06	0.34 ± 0.07	0.64 ± 0.01	0.79 ± 0.02	0.13 ± 0.03	0.38 ± 0.04	0.75 ± 0.19	0.73 ± 0.07
Vanillic acid	1.63 ± 0.01	0.76 ± 0.00	0.86 ± 0.06	0.76 ± 0.04	0.77 ± 0.00	0.86 ± 0.09	0.70 ± 0.05	0.89 ± 0.01	0.91 ± 0.03	—	—	—	0.63 ± 0.01	—	—	—	0.61 ± 0.01
Rutin	3.81 ± 0.11	1.51 ± 0.15	1.28 ± 0.01	1.86 ± 0.23	2.04 ± 0.13	1.97 ± 0.20	1.36 ± 0.01	1.93 ± 0.15	2.09 ± 0.28	0.82 ± 0.08	1.01 ± 0.10	1.34 ± 0.01	1.35 ± 0.05	0.58 ± 0.07	0.79 ± 0.14	0.97 ± 0.14	0.85 ± 0.01
Myricetin	6.92 ± 0.35	—[1]	3.38 ± 0.15	2.62 ± 0.00	3.77 ± 0.29	—	4.29 ± 0.41	4.05 ± 0.01	4.90 ± 0.37	—	—	—	—	—	—	—	—
Syringic acid	4.60 ± 0.40	—	—	—	0.85 ± 0.11	—	0.89 ± 0.00	1.63 ± 0.13	2.20 ± 0.02	—	0.07 ± 0.00	0.63 ± 0.07	0.69 ± 0.02	—	1.17 ± 0.56	1.24 ± 0.02	1.20 ± 0.09
trans-3-Hydroxy cinnamic acid	8.48 ± 0.08	0.36 ± 0.01	1.41 ± 0.03	1.33 ± 0.03	1.53 ± 0.04	0.45 ± 0.11	0.92 ± 0.05	1.01 ± 0.03	1.36 ± 0.02	0.86 ± 0.01	0.67 ± 0.01	1.03 ± 0.07	1.14 ± 0.08	0.24 ± 0.10	0.45 ± 0.08	0.60 ± 0.03	0.58 ± 0.04
2-Hydroxy cinnamic acid	1.52 ± 1.91	0.84 ± 0.01	0.68 ± 0.03	0.70 ± 0.03	0.71 ± 0.00	1.10 ± 0.01	0.52 ± 0.02	0.90 ± 0.09	0.97 ± 0.02	0.67 ± 0.06	0.41 ± 0.01	0.23 ± 0.00	0.32 ± 0.03	0.21 ± 0.01	0.27 ± 0.04	0.65 ± 0.13	0.51 ± 0.07
Naringin	4.66 ± 0.16	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Naringenin	1.48 ± 0.00	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
p-Coumaric acid	10.65 ± 0.18	1.05 ± 0.03	1.16 ± 0.02	1.88 ± 0.02	2.99 ± 0.03	1.79 ± 0.01	1.81 ± 0.04	3.70 ± 0.03	4.74 ± 0.03	0.69 ± 0.05	0.43 ± 0.06	0.83 ± 0.04	1.42 ± 0.01	0.47 ± 0.06	0.96 ± 0.02	1.49 ± 0.17	1.89 ± 0.08
Ferulic acid	1.88 ± 0.13	4.82 ± 0.01	4.70 ± 0.02	4.48 ± 0.10	5.16 ± 0.08	6.55 ± 0.04	4.04 ± 0.15	5.57 ± 0.13	5.22 ± 0.07	1.31 ± 0.02	1.66 ± 0.01	2.34 ± 0.08	3.53 ± 0.11	1.36 ± 0.01	1.51 ± 0.03	1.78 ± 0.12	1.94 ± 0.02
Sinapinic acid	4.18 ± 0.08	0.24 ± 0.04	0.82 ± 0.05	0.71 ± 0.08	0.93 ± 0.07	0.29 ± 0.12	0.52 ± 0.12	0.61 ± 0.18	0.81 ± 0.04	0.30 ± 0.11	0.31 ± 0.22	0.60 ± 0.06	0.45 ± 0.40	—	0.26 ± 0.07	0.52 ± 0.12	0.48 ± 0.03
Total	55.78 ± 3.36	9.62 ± 0.12	14.83 ± 0.10	14.82 ± 0.40	19.55 ± 0.44	13.17 ± 0.32	15.42 ± 0.21	20.96 ± 0.09	24.29 ± 0.40	4.73 ± 0.22	4.91 ± 0.33	7.63 ± 0.05	10.32 ± 0.62	2.99 ± 0.08	5.78 ± 0.81	7.99 ± 0.61	8.81 ± 0.07

[1]Not detected.

Table 5: The phenolic acid contents of ethanolic extracts of cooked mixtures of rice with various proportions of adzuki beans (*V. angularis* var. nipponensis cv. Geomguseul).

Mixing ratio of adzuki bean (%)		Only water								Added 10% fermented alcohol							
		Normal rice cooker				High-pressure rice cooker				Normal rice cooker				High-pressure rice cooker			
Phenolic compound ($\mu g\ g^{-1}$)	Raw material (Geomguseμl)	5	10	15	20	5	10	15	20	5	10	15	20	5	10	15	20
4-Hydroxybenzoic acid	5.50 ± 0.03	0.36 ± 0.00	0.37 ± 0.04	0.55 ± 0.04	0.84 ± 0.01	0.29 ± 0.01	0.56 ± 0.00	0.65 ± 0.03	1.26 ± 0.04	0.16 ± 0.02	0.30 ± 0.04	0.60 ± 0.03	0.91 ± 0.01	0.22 ± 0.06	0.26 ± 0.04	0.73 ± 0.01	1.23 ± 0.06
Vanillic acid	1.87 ± 0.08	—[1]	0.78 ± 0.05	0.70 ± 0.05	0.75 ± 0.03	0.76 ± 0.07	0.75 ± 0.03	0.88 ± 0.05	0.98 ± 0.15	0.81 ± 0.03	—	—	—	—	—	—	—
Rutin	4.89 ± 0.95	0.92 ± 0.13	1.90 ± 0.15	2.31 ± 0.11	2.55 ± 0.01	2.10 ± 0.42	2.13 ± 0.12	2.38 ± 0.10	2.90 ± 0.09	0.98 ± 0.01	1.49 ± 0.05	1.94 ± 0.05	2.14 ± 0.02	0.76 ± 0.10	1.66 ± 0.09	2.04 ± 0.05	2.02 ± 0.02
Myricetin	8.99 ± 0.39	—	—	3.08 ± 0.00	3.46 ± 0.07	—	3.24 ± 0.23	3.50 ± 0.05	5.13 ± 0.22	—	—	—	—	—	—	—	—
Gallic acid	2.79 ± 0.14	—	—	—	2.86 ± 0.51	—	—	1.80 ± 0.17	1.01 ± 0.16	—	—	—	0.14 ± 0.00	—	—	0.11 ± 0.08	1.22 ± 0.02
Syringic acid	16.90 ± 1.54	—	1.51 ± 0.08	3.18 ± 0.15	2.70 ± 0.16	—	1.15 ± 0.15	2.27 ± 0.05	2.50 ± 0.19	0.70 ± 0.04	1.34 ± 0.12	2.55 ± 0.06	3.49 ± 0.03	0.84 ± 0.41	0.78 ± 0.09	1.95 ± 0.01	2.68 ± 0.10
trans-3-Hydroxy cinnamic acid	8.02 ± 0.20	0.31 ± 0.00	1.37 ± 0.14	0.89 ± 0.04	1.80 ± 0.02	0.49 ± 0.03	1.04 ± 0.05	0.87 ± 0.01	1.94 ± 0.07	0.62 ± 0.06	0.53 ± 0.02	1.36 ± 0.08	1.55 ± 0.01	0.89 ± 0.00	0.31 ± 0.03	0.85 ± 0.04	1.20 ± 0.03
2-Hydroxy cinnamic acid	2.87 ± 1.19	0.59 ± 0.02	0.93 ± 0.07	0.40 ± 0.00	0.99 ± 0.17	1.22 ± 0.03	0.97 ± 0.06	0.41 ± 0.04	1.29 ± 0.07	0.35 ± 0.04	0.73 ± 0.09	0.83 ± 0.19	0.98 ± 0.03	0.23 ± 0.01	0.63 ± 0.06	0.78 ± 0.04	1.06 ± 0.02
Naringin	4.83 ± 0.29	—	1.07 ± 0.32	—	—	—	0.70 ± 0.04	—	1.95 ± 0.03	—	—	—	—	—	—	—	—
Cinnamic acid	3.35 ± 0.12	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Naringenin	4.23 ± 0.07	—	—	—	1.01 ± 0.12	—	—	0.54 ± 0.03	0.67 ± 0.10	—	—	—	0.49 ± 0.04	—	—	0.55 ± 0.04	1.41 ± 0.08
p-Coumaric acid	4.60 ± 0.09	0.54 ± 0.02	0.73 ± 0.03	0.95 ± 0.02	1.05 ± 0.01	1.00 ± 0.11	1.28 ± 0.00	1.59 ± 0.07	2.18 ± 0.04	0.11 ± 0.04	0.52 ± 0.04	0.59 ± 0.01	0.78 ± 0.03	0.90 ± 0.07	0.14 ± 0.06	0.67 ± 0.04	0.88 ± 0.05
Ferulic acid	5.16 ± 0.18	6.23 ± 0.04	4.97 ± 0.21	5.17 ± 0.01	5.41 ± 0.03	6.29 ± 0.09	6.16 ± 0.06	5.68 ± 0.01	6.52 ± 0.07	1.18 ± 0.10	2.21 ± 0.22	2.56 ± 0.08	5.82 ± 0.12	0.95 ± 0.07	2.28 ± 0.12	2.54 ± 0.01	6.01 ± 0.04
Sinapinic acid	4.34 ± 0.06	0.24 ± 0.03	0.80 ± 0.14	0.59 ± 0.02	1.17 ± 0.03	0.24 ± 0.03	0.66 ± 0.14	0.60 ± 0.18	1.20 ± 0.01	0.37 ± 0.02	0.51 ± 0.11	0.84 ± 0.12	0.95 ± 0.02	0.53 ± 0.02	0.14 ± 0.02	0.31 ± 0.02	0.44 ± 0.37
Total	78.35 ± 0.95	9.19 ± 0.14	14.44 ± 0.36	17.81 ± 0.37	24.58 ± 0.36	12.39 ± 0.61	18.62 ± 0.71	21.18 ± 0.47	29.54 ± 0.38	5.30 ± 0.07	7.63 ± 0.10	11.27 ± 0.32	17.25 ± 0.13	5.32 ± 0.71	6.19 ± 0.14	10.52 ± 0.06	18.15 ± 0.20

[1]Not detected.

Table 6: DPPH- and ABTS-radical-scavenging activities of the ethanolic extracts with different mixing ratios of adzuki bean (*Vigna angularis* var. nipponensis cv. Arari and cv. Geomguseul) and cooking methods.

Variety	Mixing ratio of adzuki bean (%)	DPPH radical-scavenging activity (mg trolox equivalents/100 g sample)				ABTS radical-scavenging activity (mg trolox equivalents/100 g sample)			
		GRC[1] (water)	HPRC[2] (water)	GRC (10% alcohol)	HPRC (10% alcohol)	GRC (water)	HPRC (water)	GRC (10% alcohol)	HPRC (10% alcohol)
Arari	0	3.1 ± 0.0^{e}[3]	3.7 ± 0.0^{e}	3.7 ± 0.1^{e}	3.2 ± 0.1^{e}	6.4 ± 0.4^{e}	5.0 ± 0.2^{e}	5.7 ± 0.2^{e}	6.2 ± 0.6^{e}
	5	7.2 ± 0.5^{d}	5.8 ± 0.8^{d}	10.0 ± 0.5^{d}	9.7 ± 0.4^{d}	17.9 ± 0.2^{d}	17.5 ± 0.2^{d}	19.0 ± 0.1^{d}	18.0 ± 0.3^{d}
	10	15.2 ± 0.5^{c}	14.4 ± 0.4^{c}	18.5 ± 0.3^{c}	17.0 ± 0.3^{c}	30.1 ± 0.4^{c}	28.8 ± 0.3^{c}	32.2 ± 0.2^{c}	29.9 ± 0.1^{c}
	15	24.4 ± 1.6^{b}	20.9 ± 1.4^{b}	26.8 ± 0.2^{b}	24.4 ± 0.5^{b}	41.5 ± 0.3^{b}	37.8 ± 0.3^{b}	42.8 ± 0.3^{b}	39.6 ± 0.2^{b}
	20	29.0 ± 1.5^{a}	27.7 ± 1.4^{a}	33.5 ± 0.1^{a}	31.4 ± 0.2^{a}	48.1 ± 0.4^{a}	46.0 ± 0.2^{a}	49.9 ± 0.1^{a}	48.5 ± 0.2^{a}
Geomguseul	0	3.1 ± 0.0^{e}	3.7 ± 0.0^{e}	3.7 ± 0.1^{e}	3.2 ± 0.1^{e}	6.4 ± 0.4^{e}	5.0 ± 0.2^{e}	5.7 ± 0.2^{e}	6.2 ± 0.6^{e}
	5	8.4 ± 2.1^{d}	7.9 ± 2.1^{d}	9.1 ± 1.9^{d}	9.0 ± 1.6^{d}	18.3 ± 0.1^{d}	17.7 ± 0.0^{d}	20.1 ± 0.2^{d}	18.9 ± 0.3^{d}
	10	15.6 ± 0.5^{c}	13.7 ± 0.5^{c}	18.1 ± 0.2^{c}	16.1 ± 0.3^{c}	31.4 ± 0.2^{c}	28.9 ± 0.2^{c}	32.2 ± 0.2^{c}	29.2 ± 0.1^{c}
	15	22.8 ± 0.3^{b}	20.5 ± 0.4^{b}	26.1 ± 0.2^{b}	22.2 ± 0.2^{b}	41.0 ± 0.5^{b}	37.6 ± 0.2^{b}	42.4 ± 0.3^{b}	38.2 ± 0.3^{b}
	20	27.7 ± 1.7^{a}	29.4 ± 1.6^{a}	34.0 ± 0.6^{a}	29.2 ± 0.3^{a}	46.8 ± 0.2^{a}	45.5 ± 0.2^{a}	51.5 ± 0.1^{a}	46.6 ± 0.1^{a}

[1]Normal rice cooker; [2]high-pressure rice cooker. All values are expressed as the mean ± SD of triplicate determinations; [3]all values are expressed as the mean ± SD of triplicate determinations. Means with different superscripts within a column (a–f) are significantly different at $p < 0.05$ by one-way analysis of variance (ANOVA) using Duncan's multiple range test.

173.0 ± 1.0 and 171.4 ± 5.8 μg CE/g (normal and high-pressure cooking); the figures after cooking with 10% (v/v) alcohol were 162.6 ± 1.5 and 178.5 ± 4.8 μg CE/g, respectively (Table 3). The total flavonoid contents of mixtures with Geomguseul beans (5–20%) cooked in water in normal and high-pressure cookers were 39.8–141.0 and 48.7–168.5 μg CE/g; and those of mixtures cooked in 10% (v/v) alcohol 35.3–140.1 and 39.9–137.3 μg CE/g respectively (Table 3). Flavonoids are composed principally of anthocyanidins, flavonols, flavones, cathechins, and flavanones. Depending on the structure, specific flavonoids exert various physiological actions, including antioxidation and antibacterial activities [19]. Plants contain many antioxidants, principally as covalently bound insoluble polymers [28]. Therefore, heat may break down cell walls and liberate antioxidant compounds from insoluble components of adzuki beans, increasing the bioaccessible pool thereof [20]. We confirmed that addition of adzuki beans proportionately increased antioxidant levels in cooked rice, but the cooking method must also be considered because this affects texture (and thus acceptability).

3.4. Individual Phenolic Levels in Cooked Rice/Adzuki Bean Mixtures. The phenolic acid compositions of various rice/adzuki bean mixtures, by cooking method, are shown in Tables 4 and 5. The phenolic acids of rice/Arari bean mixtures were 4-hydroxybenzoic acid (5.98 μg/g), vanillic acid (1.63 μg/g), rutin (3.81 μg/g), myricetin (6.92 μg/g), syringic acid (4.60 μg/g), *trans*-3-hydroxycinnamic acid (8.48 μg/g), 2-hydroxy cinnamic acid (1.52 μg/g), naringin (4.66 μg/g), naringenin (1.48 μg/g), *p*-coumaric acid (10.65 μg/g), ferulic acid (1.88 μg/g), and sinapinic acid (4.18 μg/g) (Table 4). The total phenolic acid content of Arari beans was 55.78 μg/g. The predominant phenolic acid was *p*-coumaric acid; the levels of samples cooked in water were 1.05–2.99 and 1.79–4.74 μg/g (normal and high-pressure rice cookers, resp.); the figures for mixtures cooked in 10% (v/v) alcohol were 0.43–1.42 and 0.47–1.89 μg/g, respectively. The *trans*-3-hydroxycinnamic acid levels of water-cooked samples were 0.36–1.53 and 0.45–1.36 μg/g (normal and high-pressure rice cookers, resp.); the figures for mixtures cooked in 10% (v/v) alcohol were 0.67–1.14 and 0.24–0.60 μg/g, respectively. Neither naringin nor naringenin was detected in any sample containing Arari beans. The total phenolic acid levels in rice/Arari bean mixtures cooked in water were 9.62–19.55 and 13.17–24.29 μg/g (normal and high-pressure rice cookers, resp.); the figures for mixtures cooked in 10% (v/v) alcohol were 4.73–10.32 and 2.99–8.81 μg/g, respectively. Overall, phenolic acid levels were higher in samples cooked in plain water.

The phenolic acids of Geomguseul beans included 4-hydroxybenzoic acid (5.50 μg/g), vanillic acid (1.87 μg/g), rutin (4.89 μg/g), myricetin (8.99 μg/g), gallic acid (2.79 μg/g), syringic acid (16.90 μg/g), *trans*-3-hydroxycinnamic acid (8.02 μg/g), 2-hydroxy cinnamic acid (2.87 μg/g), naringin (4.83 μg/g), cinnamic acid (3.35 μg/g), naringenin (4.23 μg/g), *p*-coumaric acid (4.60 μg/g), ferulic acid (5.16 μg/g), and sinapinic acid (4.34 μg/g) (Table 5). The total phenolic acid content was 78.35 μg/g. The predominant phenolic acid was syringic acid; the levels in mixtures cooked in water were 0–3.18 and 0–2.50 μg/g (normal and high-pressure rice cookers, resp.); the figures for mixtures cooked in 10% (v/v) alcohol were 0.70–3.49 and 0.78–2.68 μg/g, respectively. The *trans*-3-hydroxycinnamic acid levels in mixtures cooked in water were 0.31–1.80 and 0.49–1.94 μg/g (normal and high-pressure rice cookers, resp.); the figures for mixtures cooked in 10% (v/v) alcohol were 0.53–1.55 and 0.31–1.20 μg/g, respectively. Cinnamic acid was not detected in cooked rice/Geomguseul bean mixtures. The total phenolic acid contents in cooked rice/Geomguseul bean mixtures cooked in water were 9.19–24.58 and 12.39–29.54 μg/g (normal and high-pressure rice cookers, resp.); the figures for mixtures cooked in 10% (v/v) alcohol were 5.30–17.25 and 5.32–18.15 μg/g, respectively. Overall, the phenolic acid content was higher in mixtures cooked in water. High temperatures (>100°C) were reported to destroy the flavonoids of citrus peel [29]. However, Lou et al. [30] found that phenolic levels in hot water extracts of the peel of immature

calamondins increased after heating at 150°C for 1.5 h. Release of naringin, tangeretin, ferulic acid, p-coumaric acid, and gallic acid was enhanced. Kim et al. [31] reported that the chlorogenic, ferulic, caffeic, and cinnamic acid contents of small black soybeans increased after roasting.

3.5. Radical-Scavenging Activities of Cooked Rice/Adzuki Bean Mixtures. DPPH radical-scavenging activity is used to measure the electron-donating capacities of antioxidants; the intensity of a dark purple color is reduced in the presence of antioxidants, such as ascorbic acid, tocophenol, polyhydroxy aromatic compounds, and aromatic amines [32]. The stable DPPH radical, with a maximum absorption at 515 nm, is widely used to evaluate the free radical-scavenging activity of hydrogen-donating antioxidants of plant extracts [33]. The DPPH radical-scavenging activities of cooked rice/adzuki bean mixtures were compared with that of the standard Trolox (Table 6); activity increased as the adzuki bean proportion rose. The activity of plain rice ranged from 3.1–3.7 mg TE/100 g by the cooking method. The activities of mixtures of rice/Arari beans (20% w/w) cooked in water averaged 29.0 ± 1.5 and 27.7 ± 1.4 mg TE/100 g (normal and high-pressure rice cookers, resp.); the figures for mixtures cooked in 10% (v/v) alcohol were 33.5 ± 0.1 and 31.4 ± 0.2 mg TE 100/g, respectively (Table 6). The activity of rice/Geomguseul bean mixtures (5–20% w/w) cooked in water were 8.4–27.7 and 7.9–29.4 mg TE/100 g (normal and high-pressure rice cookers, resp.); the figures for mixtures cooked in 10% (v/v) alcohol were 9.1–34.0 and 9.0–29.2 mg TE/100 g, respectively (Table 6).

ABTS radical-scavenging activity was measured using the method of Kim et al. [34]. When ABTS is mixed with potassium persulfate in the dark, $ABTS^{+\cdot}$ is generated and then consumed by antioxidants in the extract. Thus, the cyan color is lost. The ABTS method is widely used to measure the radical-scavenging activities of hydrogen-donating and chain-breaking antioxidants of many plant extracts [35]. The ABTS radical-scavenging activities of cooked rice/adzuki bean mixtures were compared with that of the standard Trolox (Table 6). The radical-scavenging activity increased as the adzuki bean proportion rose. The activity of plain rice varied by the cooking method from 5.0–6.4 mg TE/100 g. The activity of rice/Arari bean mixtures (20% w/w) cooked in water in a normal and a high-pressure rice cooker averaged 48.1 ± 0.4 and 46.0 ± 0.2 mg TE/100 g; the figures for mixtures prepared with 10% (v/v) alcohol were 49.9 ± 0.1 and 48.5 ± 0.2 mg TE/100 g, respectively (Table 6); and the figures for Geomguseul beans (5–20% w/w) cooked in water and 10% (v/v) alcohol were 18.3–46.8 and 17.7–45.5; and 20.1–51.5 and 18.9–46.6 mg TE/100 g, respectively (Table 6). Antioxidants inhibit lipid oxidation, slow aging, and inhibit disease [16]. Recently, it was found that prolonged heat treatment enhanced the antioxidant activities of tomato and coffee [36]. Browning and antioxidant activities increased as heating/roasting times increased. Over the past decade, many studies have measured antioxidant activities after heat treatment; heated products exhibited elevated chain-breaking and oxygen-scavenging activities [37]. When rice/adzuki bean mixes were cooked in normal cookers, radical-scavenging activity was high; such cookers can thus be used for industrial applications.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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