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Effect of Cooking Methods on Phenolic Compounds and their Radical Scavenging Activity of Cooked Mixed Grain Rice/Sorghum Mixtures

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Abstract

This study explored the optimal preparation and the antioxidant levels of rice and sorghum mixtures. We compared the quality and physicochemical characteristics of cooked mixtures of rice and sorghum prepared using normal and high-pressure rice cookers, with and without the addition of alcohol. The water-binding capacity and swelling power decreased upon addition of sorghum, but water solubility increased. The peak, trough, breakdown, final, and setback viscosities decreased upon addition of sorghum. The total polyphenol and flavonoid contents, and radical scavenging activity increased after addition of sorghum. The predominant phenolic acid of rice/sorghum mixtures was t-3-hydroxy cinnamic acid, naringin, p-coumaric acid and ferulic acid. The DPPH- and ABTS-radical scavenging activities increased upon addition of sorghum. Moreover, rice/sorghum mixtures prepared in a high pressure cooker, with addition of alcohol, exhibited higher antioxidant levels than other samples. We present the antioxidative properties of rice/sorghum mixtures prepared in different ways; these data will aid manufacturers.

Keywords: Sorghum (*Sorghum bicolor* L. Moench); Cooking characteristics; Phenolic compound; Antioxidant activity

Introduction

Sorghum (*Sorghum bicolor* L. Moench) is an important crop in the semiarid regions of Africa and Asia as a source of energy, protein, minerals, and vitamins [1]. Sorghum grain is an important food in developing countries of the semiarid tropics, and is also a main source of energy and protein in animal feed in both developed and developing countries [2,3]. Sorghum contains phenolic compounds in the form of phenolic acids, flavonoids, and condensed tannins, which have been shown to have antioxidant activity [4,5]. These phenolic compounds are concentrated in the outer layers of the sorghum grain (pericarp and testa) [6], and possess structural features that are favorable for radical scavenging and/or metal chelation, making them effective antioxidants.

Recent studies have shown that thermally processed foods, especially fruits and vegetables, have higher biological activity due to changes in various compounds during heat treatment [7]. The latest studies have reported that heat treatment increases total polyphenolic and flavonoid contents, and antioxidant activities in garlic [8], pears [9], tomatoes [10], oranges [11], and shiitake mushroom [12]. Also, recent epidemiological studies have suggested that increased consumption of whole grains, fruits, and vegetables is associated with reduced risk of chronic diseases [13]. This association might be attributed to natural antioxidants such as vitamin C, tocopherol, carotenoids, polyphenolics, and flavonoids, which prevent free-radical damage [14].

The purpose of this study was to investigate the cooking characteristics and antioxidant activity of cooked-rice with different mixing ratio of sorghum and cooking methods for increase the eating quality characteristic and functional component content of cooked-rice with added sorghum. This is to be used as a basic data for calculating the mixing ratio of cereals. In addition, the effect of addition of fermented alcohol on heat treatment of cereals was investigated in order to improve the functionality of cooked-rice added with sorghum [15].

Materials and Methods

Sample preparation

We used sorghum of the cultivars *Sorghum bicolor* L. Moench cv. Nampungchal (glutinous), and Donganme (non-glutinous), and rice of the cultivar *Oryza sativa* cv. Samkwang rice. The sorghum and Samkwang rice cultivars were grown at the National Institute of Crop Science, Rural Development Administration, Miryang, South Korea during the 2015 growing season. The rice cultivar Samkwang is a high-quality taste-rich rice cultivated in 2003 by the National Institute of Crop Science. 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). The sorghum was prepared using a milling machine (SY2009-MMCMT, Ssangyong Ltd.). The amylose levels of the rice cultivar Samkwang, sorghum cultivar Nampungchal and Donganme were 22.35, 5.92 and 32.00 g/100 g, respectively (data not showed). 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.

Analysis of pasting characteristics

The pasting characteristics of rice prepared with different proportions of sorghum (sorghum at 0, 5, 10, 15 and 20% w/w) were measured using the methods of Kim et al. [16] and a rapid viscosity analyzer (Model RVA-3D; Newport Scientific, Warriewood, NSW,

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Australia). Rice/sorghum 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; 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. All experiments were performed in triplicate.

Analysis of water binding capacity, swelling power and water solubility

The water-binding capacities of various rice/sorghum mixtures (sorghum 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 [17]. Supernatants were removed by centrifugation at 1,500 × g for 10 min, and the weights of precipitated powders 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 ± 1°C 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 also weighed [18]. All experiments were performed in triplicate.

Cooking methods

Rice/sorghum mixtures were prepared with 0, 5, 10, 15 and 20% w/w sorghum, washed three times, soaked in water (120 mL) 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. The functionality of sorghum cereal improves on addition of alcohol [15]. The drained water were used for cooking which is to minimize the loss of useful components. We used normal (Cuckoo CR-0671V, Seoul, Korea) and high-pressure (Cuckoo EHS035FW) rice cookers. The mixtures were placed in a cooking container of a rice cooker and then cooked. The mixtures were automatically boiled or steamed for 50 min. The cooked rice was used as a sample for phenolic compounds and radical scavenging activity analysis.

Determination of phenolic compounds

We measured the levels of phenolics 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 [19]. 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 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 $AlCl_3.6H_2O$ (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 measured. The results are expressed as µg catechin (Sigma-Aldrich) equivalents per g of sample. All experiments were performed in triplicate.

Measurement of individual phenolic acid levels

The phenolic acid composition of each extract was determined via HPLC as described by Kim et al. [20] with slight modifications. We 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, *t*-3-hydroxy cinnamic 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 (J.T. Baker, Phillipsburg, NJ, USA). 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. All experiments were performed in triplicate.

Measurement of DPPH- and ABTS-radical scavenging activities

DPPH- and ABTS-radical scavenging activities were measured according to Woo et al. [19], 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 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, $\varepsilon = 3.6 \times 10^4$ mol^{-1} 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. All experiments were performed in triplicate.

Statistical analysis

All data are expressed as means ± standard deviations (SDs). Significant differences among treatments was 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.

Results and Discussion

Pasting characteristics

We determined pasting characteristics by sorghum proportion; we measured peak, trough, breakdown, final, and setback viscosities (Table 1). As the sorghum 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 [21]. The breakdown viscosity of mixtures containing both Nampungchal and Donganme sorghums (5–20% w/w) were 66.03–69.03 and 52.08–65.47 RVU, respectively, and did not vary by the sorghum proportion. The final viscosity was measured after completion of both heating and cooling; starch particles (such as amylose) then recombine to increase viscosity [21]. As the sorghum proportion increased, the viscosities of mixtures containing both Nampungchal and Donganme sorghums fell to 209.11–237.28 and 223.42–241.61 RVU, respectively. The setback viscosity reflects starch aging; the higher the value, the more rapid the aging [21]. 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. We found an inverse relationship between the proportion of sorghums and setback viscosity. Thus, the higher the bean proportion, the slower the aging.

Water characteristics

In Table 2, water binding capacity increased from 132.71 (sorghum 5%) to 141.63 (sorghum 20%). The water-binding capacities of mixtures of rice with Nampungchal and Donganme sorghums (5–20% w/w) were 139.16–144.50 and 144.58–157.97%, 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 [22]. 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 [23]. Following the addition of Nampungchal and Donganme sorghums (5–20% w/w) to rice, water solubility increased significantly from 6.77 to 7.94% and 5.22 to 6.54%, respectively, and swelling power decreased significantly from 114.81 to 99.05% and 164.90 to 140.16%, respectively, as the sorghum 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 [16]. 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 [24]. 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 [25].

Phenolic compounds of rice cooked with different proportions of sorghums

Phenolics are the major antioxidants of fruits, vegetables, and grains [26]. Therefore, we measured polyphenolic levels and their antioxidant contributions. The polyphenolic levels of rice/sorghum mixtures with different sorghum proportions are shown, by cooking method in Figure 1. The total polyphenol content of plain rice varied by the cooking method from 161.57–177.50 µg GAE/g. The total polyphenol contents of mixes with 20% (w/w) Nampungchal sorghums cooked in water averaged 436.05 ± 10.28 and 427.33 ± 6.73 µg GAE/g (normal and high-pressure cooking, respectively); the figures for the same mixtures cooked with 10%

Variety	Mixing ratio of sorghum (%)	Peak viscosity (RVU[1]))	Trough viscosity (RVU)	Break down (RVU)	Final viscosity (RVU)	Set back (RVU)
Nampungchal	0	195.00 ± 1.38[a 2)]	127.89 ± 2.49[a]	67.11 ± 3.88[b]	251.97 ± 2.30[a]	56.97 ± 3.64[b]
	5	187.50 ± 0.17[b]	121.47 ± 1.89[b]	66.03 ± 1.96[a]	237.28 ± 1.68[b]	49.78 ± 1.75[b]
	10	185.53 ± 1.51[b]	116.50 ± 0.38[c]	69.03 ± 1.14[a]	228.97 ± 2.32[c]	43.45 ± 2.04[c]
	15	181.45 ± 0.91[c]	114.44 ± 1.02[c]	67.00 ± 0.54[a]	221.25 ± 0.83[d]	39.81 ± 1.73[c]
	20	176.91 ± 2.04[d]	108.42 ± 1.32[d]	68.50 ± 2.65[a]	209.11 ± 1.75[e]	32.20 ± 2.06[d]
Donganme	0	195.00 ± 1.38[a]	127.89 ± 2.49[a]	67.11 ± 3.88[a]	251.97 ± 2.30[a]	56.97 ± 3.64[a]
	5	183.50 ± 0.58[b]	118.03 ± 0.63[b]	65.47 ± 0.29[a]	241.61 ± 4.06[b]	58.11 ± 4.65[a]
	10	178.33 ±1.66[c]	117.47 ± 1.16[b]	60.89 ± 2.61[b]	234.89 ± 3.51[c]	56.56 ± 4.66[a]
	15	170.14 ± 0.10[d]	114.53 ± 0.63[c]	55.61 ± 0.68[c]	228.39 ± 2.71[d]	58.25 ± 2.73[a]
	20	165.75 ± 1.59[e]	113.67 ± 0.65[c]	52.08 ± 1.06[c]	223.42 ± 0.44[e]	57.67 ± 1.53[a]

Note: 1)Rapid Visco Units. 2)All values are means ± SDs of those of triplicate determinations. Means bearing different superscripts within a column (a–e) differed significantly at $p<0.05$ by one-way analysis of variance (ANOVA) using Duncan's multiple range test.

Table 1: Pasting characteristics of with mixing ratio of sorghum (*S. bicolor* L. Moench cv. Nampungchal (glutinous), and Donganme (non-glutinous)).

Variety	Mixing ratio of sorghum (%)	Water binding capacity (%)	Water solubility index (%)	Swelling power (%)
Nampungchal	0	187.31 ± 2.46[a1]	5.35 ± 0.16[b]	162.37 ± 4.04[a]
	5	132.71 ± 1.88[c]	6.77 ± 0.13a[b]	114.81 ± 2.84[b]
	10	139.16 ± 4.57[bc]	7.21 ± 0.14[a]	106.28 ± 4.55[b]
	15	140.81 ± 4.85[b]	7.30 ± 0.58[a]	103.47 ± 6.70[b]
	20	141.63 ± 3.66[b]	7.94 ± 0.43[a]	99.05 ± 7.19[b]
Donganme	0	187.31 ± 2.46[a]	5.35 ± 0.16[c]	162.37 ± 4.04[ab]
	5	157.97 ± 7.87[b]	5.22 ± 0.06[c]	164.90 ± 4.03[a]
	10	152.90 ± 4.62b[c]	5.58 ± 0.15[bc]	153.47 ± 9.21[ab]
	15	145.92 ± 5.55[c]	5.90 ± 0.19[b]	151.11 ± 4.47[bc]
	20	144.58 ± 3.68[c]	6.54 ± 0.15[a]	140.16 ± 1.88[c]

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

Table 2: Water binding capacity, water solubility index, and swelling power of with mixing ratio of sorghum (*S. bicolor* L. Moench cv. Nampungchal (glutinous), and Donganme (non-glutinous)).

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(v/v) alcohol were 469.20 ± 2.92 and 464.06 ± 7.68 µg GAE/g (Figure 1A). The figures for rice/sorghum mixtures with 5–20% (w/w) Donganme sorghums were 199.96–360.93 and 168.33–378.13 µg GAE/g (water; normal and high-pressure cooking), and 220.05–379.85 and 220.70–391.35 µg GAE/g (10% [v/v] alcohol; normal and high- pressure cooking), respectively (Figure 1B). Of all cereal antioxidants, polyphenolics are the most powerful, as the phenolic ring stabilizes free radicals [18].

The total flavonoid content of mixtures increased as the sorghum proportion increased (Figure 2). The total flavonoid contents of plain rice were 11.99–18.84 and 9.63–11.54 µg CE/g depending on the cooking method (normal and high-pressure, respectively). The total flavonoid contents of rice with 20% Nampungchal sorghums cooked in water were 160.70 ± 2.55 and 161.05 ± 2.73 µg CE/g (normal and high-pressure cooking); the figures after cooking with 10% (v/v) alcohol were 200.93 ± 5.34 and 209.90 ± 4.09 µg CE/g, respectively (Figure 2A). The total flavonoid contents of mixtures with Donganme sorghums (5–20%) cooked in water in normal and high-pressure cookers were 36.14–125.60 and 43.07–136.75 µg CE/g; and those of mixtures cooked in 10% (v/v) alcohol 48.48–145.70 and 46.53–153.85 µg CE/g respectively (Figure 2B). 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 [18]. Plants contain many antioxidants, principally as covalently bound insoluble polymers [11]. Therefore, heat may break down cell walls and liberate antioxidant compounds from insoluble components of sorghums, increasing the bioaccessible pool thereof [19]. We confirmed that addition of sorghums proportionately increased antioxidant levels in cooked rice, but the cooking method must also be considered because this affects texture (and thus acceptability).

Individual phenolic levels in cooked rice/sorghum mixtures

The phenolic acid compositions of various rice/sorghums mixtures, by cooking method, are shown in Tables 3 and 4. The phenolic acids of Nampungchal sorghum were 4-hydroxybenzoic acid (0.82 µg/g), vanillic acid (15.73 µg/g), rutin (6.27 µg/g), protocatechuic acid (2.51 µg/g), quercertin (6.65 µg/g), syringic acid (5.86 µg/g), t-3-hydroxy cinnamic acid (87.32 µg/g), naringin (27.46 µg/g), cinnamic acid (5.57 µg/g), naringenin (8.42 µg/g), chlorogenic acid (8.58 µg/g), caffeic acid (20.00 µg/g), p-coumaric acid (6.22 µg/g), ferulic acid (7.04 µg/g), and sinapinic acid (5.60 µg/g) (Table 3). The total phenolic acid content of Nampungchal sorghums was 214.06 µg/g. The predominant phenolic acid was t-3-hydroxy cinnamic acid; the levels of samples cooked in water were 8.43–45.76 and 7.11–40.99 µg/g (normal and high-pressure rice cookers, respectively); the figures for mixtures cooked in 10% (v/v) alcohol were 9.24–38.10 and 7.93–36.43 µg/g, respectively. The naringin levels of water-cooked samples were 2.45–13.26 and 2.64–13.05 µg/g (normal and high-pressure rice cookers, respectively); the figures for mixtures cooked in 10% (v/v) alcohol were 3.22–10.89 and 1.16–11.61 µg/g, respectively. The vanillic acid, p-coumaric acid and ferulic acid levels of rice/Nampungchal sorghum mixtures (5–20% w/w) were 0.21–3.02, 0.80–6.38 and 1.24–3.52 µg/g, respectively. The total phenolic acid levels in rice/Nampungchal sorghum mixtures cooked in water were 21.47–102.61 and 20.42–107.11 µg/g (normal

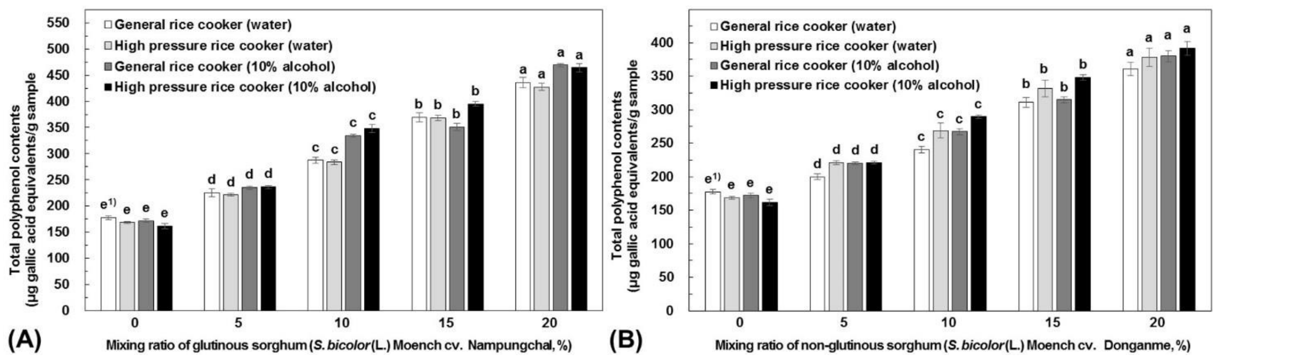

Figure 1: Total polyphenol contents on the ethanolic extracts with different mixing ratio of sorghum (*Sorghum bicolor* L. Moench cv. Nampungchal (glutinous; A), and Donganme (non-glutinous; B)) and cooking methods. [1)]Any means in the same column followed by the same letter are not significantly (p<0.05) different by Duncan's multiple range test.

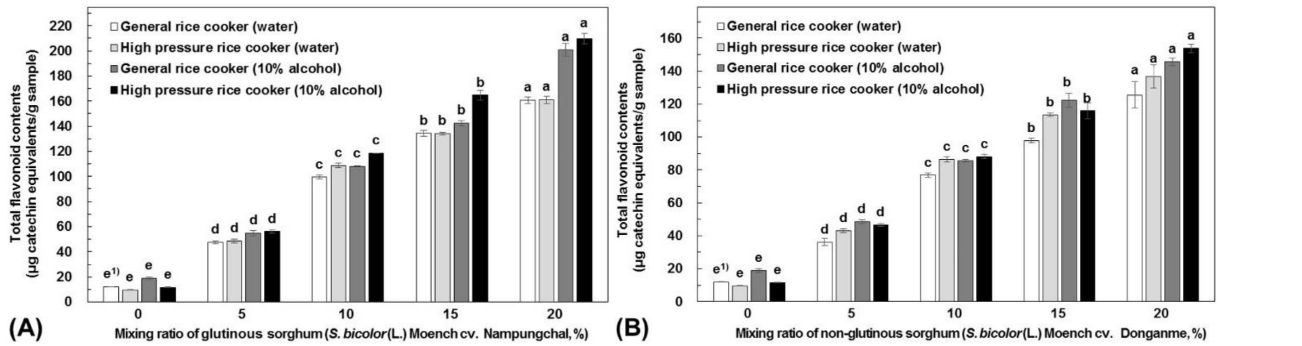

Figure 2: Total flavonoid contents on the ethanolic extracts with different mixing ratio of sorghum (*Sorghum bicolor* L. Moench cv. Nampungchal (glutinous; A), and Donganme (non-glutinous; B)) and cooking methods. [1)]Any means in the same column followed by the same letter are not significantly (p<0.05) different by Duncan's multiple range test.

Mixing ratio of sorghum (%)		Only water								Added 10% fermented alcohol							
		Normal rice cooker				High-pressure rice cooker				Normal rice cooker				High-pressure rice cooker			
Phenolic compound (μg/g)	Raw material	5	10	15	20	5	10	15	20	5	10	15	20	5	10	15	20
4-Hydroxy- benzoic acid	0.82 ± 0.04	0.54 ± 0.00	1.05 ± 0.08	2.55 ± 0.00	3.65 ± 0.04	0.65 ± 0.03	1.84 ± 0.00	2.84 ± 0.11	4.94 ± 0.05	0.74 ± 0.02	1.42 ± 0.01	1.94 ± 0.09	3.41 ± 0.14	0.83 ± 0.01	0.61 ± 0.01	8.89 ± 0.20	4.11 ± 0.21
Vanillic acid	15.73 ± 1.81	0.30 ± 0.02	0.42 ± 0.01	0.98 ± 0.01	1.68 ± 0.13	0.57 ± 0.01	0.70 ± 0.07	0.84 ± 0.01	1.54 ± 0.05	0.24 ± 0.00	0.40 ± 0.02	0.63 ± 0.05	1.34 ± 0.04	0.21 ± 0.01	0.51 ± 0.04	1.07 ± 0.18	3.02 ± 0.01
Rutin	6.27 ± 0.04	1.25 ± 0.08	2.04 ± 0.07	4.51 ± 0.04	5.11 ± 0.06	0.86 ± 0.12	2.38 ± 0.05	5.35 ± 0.02	7.09 ± 0.01	0.60 ± 0.01	2.51 ± 0.09	2.43 ± 0.11	5.00 ± 0.06	0.53 ± 0.02	1.20 ± 0.04	1.75 ± 0.03	2.52 ± 0.03
Protocatechuic acid	2.51 ± 0.13	0.30 ± 0.01	0.75 ± 0.02	1.06 ± 0.01	1.55 ± 0.08	1.53 ± 0.03	1.12 ± 0.04	1.28 ± 0.01	1.80 ± 0.01	3.90 ± 0.07	4.96 ± 0.07	5.11 ± 0.02	2.71 ± 0.04	0.46 ± 0.04	1.40 ± 0.11	2.35 ± 0.00	4.71 ± 0.03
Quercertin	6.65 ± 0.70	0.72 ± 0.00	1.01 ± 0.13	2.10 ± 0.02	4.03 ± 0.15	0.51 ± 0.05	1.15 ± 0.04	2.03 ± 0.23	4.72 ± 0.02	0.74 ± 0.04	1.24 ± 0.04	1.62 ± 0.07	2.16 ± 0.13	0.76 ± 0.02	1.44 ± 0.01	2.96 ± 0.02	3.14 ± 0.06
Syringic acid	5.86 ± 0.08	2.87 ± 0.02	3.80 ± 0.02	9.39 ± 0.07	12.18 ± 0.31	2.14 ± 0.03	6.20 ± 0.06	8.50 ± 0.03	14.54 ± 0.04	2.73 ± 0.05	6.36 ± 0.02	8.41 ± 0.05	25.60 ± 0.07	2.73 ± 0.01	6.09 ± 0.06	9.60 ± 0.21	23.17 ± 0.03
t-3-Hydroxy cinnamic acid	87.32 ± 0.25	8.43 ± 0.01	8.12 ± 0.10	36.23 ± 0.10	45.76 ± 0.25	7.11 ± 0.04	13.55 ± 0.01	22.71 ± 0.45	40.99 ± 0.04	9.24 ± 0.05	22.96 ± 0.09	28.05 ± 0.01	38.10 ± 0.76	7.93 ± 0.20	24.86 ± 0.09	30.05 ± 0.20	36.43 ± 0.88
Naringin	27.46 ± 0.10	2.45 ± 0.05	5.36 ± 0.03	9.85 ± 0.06	13.26 ± 0.02	2.64 ± 0.01	4.65 ± 0.03	7.23 ± 0.13	13.05 ± 0.12	3.22 ± 0.04	6.62 ± 0.01	8.81 ± 0.14	10.89 ± 0.41	1.16 ± 0.05	2.73 ± 0.02	3.07 ± 0.10	11.61 ± 0.14
Cinnamic acid	5.57 ± 0.20	0.31 ± 0.01	0.44 ± 0.01	1.08 ± 0.01	1.28 ± 0.01	0.37 ± 0.00	0.47 ± 0.02	0.71 ± 0.00	1.34 ± 0.05	0.29 ± 0.01	0.52 ± 0.00	0.68 ± 0.01	1.11 ± 0.02	0.33 ± 0.01	0.72 ± 0.00	1.38 ± 0.00	2.88 ± 0.00
Naringenin	8.42 ± 0.25	0.32 ± 0.01	0.48 ± 0.01	1.32 ± 0.05	1.75 ± 0.03	0.29 ± 0.05	0.72 ± 0.05	0.97 ± 0.01	1.97 ± 0.02	0.22 ± 0.05	0.50 ± 0.01	0.93 ± 0.06	1.45 ± 0.08	0.22 ± 0.00	0.29 ± 0.01	1.89 ± 0.01	2.27 ± 0.02
Chlorogenic acid	8.58 ± 0.31	0.92 ± 0.02	1.16 ± 0.06	2.96 ± 0.10	3.97 ± 0.17	0.58 ± 0.02	1.82 ± 0.05	2.57 ± 0.04	4.78 ± 0.06	0.79 ± 0.11	1.98 ± 0.10	2.99 ± 0.10	3.79 ± 0.09	0.72 ± 0.02	0.81 ± 0.00	1.02 ± 0.00	4.66 ± 0.02
Caffeic acid	20.00 ± 0.52	0.13 ± 0.01	0.20 ± 0.01	0.39 ± 0.04	0.39 ± 0.00	0.10 ± 0.01	0.18 ± 0.01	0.29 ± 0.00	0.45 ± 0.06	0.10 ± 0.01	0.31 ± 0.01	0.31 ± 0.03	0.41 ± 0.02	0.10 ± 0.00	0.17 ± 0.01	0.50 ± 0.01	1.22 ± 0.01
p-Coumaric acid	6.22 ± 0.07	1.29 ± 0.05	1.89 ± 0.02	3.91 ± 0.07	5.11 ± 0.02	1.36 ± 0.01	2.89 ± 0.04	3.81 ± 0.09	6.38 ± 0.06	1.20 ± 0.02	2.83 ± 0.00	4.14 ± 0.01	4.77 ± 0.10	0.80 ± 0.01	1.33 ± 0.00	1.48 ± 0.01	5.70 ± 0.11
Ferulic acid	6.22 ± 0.07	1.63 ± 0.02	1.68 ± 0.03	2.58 ± 0.07	2.88 ± 0.03	1.73 ± 0.02	2.38 ± 0.05	2.71 ± 0.01	3.52 ± 0.02	1.32 ± 0.02	2.17 ± 0.05	2.51 ± 0.02	2.52 ± 0.07	1.24 ± 0.03	1.35 ± 0.05	1.85 ± 0.09	3.04 ± 0.01
Sinapinic acid	5.60 ± 0.20	-[1)]	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Total	214.06 ± 2.18	21.47 ± 0.15	38.41 ± 0.40	78.92 ± 0.16	102.61 ± 0.12	20.42 ± 0.14	40.05 ± 0.04	61.84 ± 0.44	107.11 ± 0.17	25.34 ± 0.30	54.77 ± 0.01	68.56 ± 0.18	103.26 ± 1.87	18.03 ± 0.22	43.50 ± 0.10	67.86 ± 0.14	108.47 ± 1.07

Note: [1)]Not detected.

Table 3: The phenolic compounds contents in ethanolic extracts with different mixing ratio of glutinous sorghum (*Sorghum bicolor* L. Moench cv. Nampungchal).

Mixing ratio of sorghum (%)		Only water								Added 10% fermented alcohol							
		Normal rice cooker				High-pressure rice cooker				Normal rice cooker				High-pressure rice cooker			
Phenolic compound (μg/g)	Raw material	5	10	15	20	5	10	15	20	5	10	15	20	5	10	15	20
4-Hydroxy- benzoic acid	6.06 ± 0.02	0.13 ± 0.00	0.21 ± 0.01	0.57 ± 0.03	0.65 ± 0.04	0.23 ± 0.01	0.54 ± 0.01	0.81 ± 0.00	1.26 ± 0.00	0.08 ± 0.02	0.12 ± 0.01	0.34 ± 0.00	0.57 ± 0.04	0.22 ± 0.01	0.31 ± 0.03	0.56 ± 0.00	1.06 ± 0.01
Vanillic acid	2.66 ± 0.03	0.23 ± 0.01	0.23 ± 0.03	0.24 ± 0.02	0.30 ± 0.01	0.17 ± 0.00	0.23 ± 0.01	0.29 ± 0.00	0.35 ± 0.00	0.17 ± 0.01	0.20 ± 0.00	0.21 ± 0.00	0.24 ± 0.01	0.24 ± 0.00	0.30 ± 0.00	0.33 ± 0.01	0.35 ± 0.00
Rutin	7.98 ± 0.15	0.31 ± 0.01	0.44 ± 0.02	0.53 ± 0.00	0.82 ± 0.00	0.53 ± 0.02	0.57 ± 0.01	0.67 ± 0.02	0.95 ± 0.02	0.25 ± 0.01	0.58 ± 0.01	0.86 ± 0.03	1.53 ± 0.01	0.25 ± 0.01	0.82 ± 0.01	1.22 ± 0.01	1.83 ± 0.01
Myricetin	8.52 ± 0.91	0.22 ± 0.01	0.36 ± 0.03	0.44 ± 0.01	0.54 ± 0.01	0.20 ± 0.02	0.32 ± 0.00	0.71 ± 0.01	1.18 ± 0.00	0.19 ± 0.00	0.32 ± 0.00	0.46 ± 0.00	0.60 ± 0.01	0.28 ± 0.00	0.39 ± 0.01	0.51 ± 0.01	0.59 ± 0.01
Quercertin	4.25 ± 0.05	-[1)]	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Syringic acid	17.95 ± 0.03	0.44 ± 0.00	1.06 ± 0.09	1.96 ± 0.11	2.31 ± 0.02	0.58 ± 0.03	1.19 ± 0.03	1.55 ± 0.01	3.03 ± 0.04	0.37 ± 0.01	0.64 ± 0.01	1.30 ± 0.00	1.74 ± 0.00	0.59 ± 0.01	0.79 ± 0.07	1.48 ± 0.04	2.41 ± 0.05
t-3-Hydroxy cinnamic acid	454.21 ± 0.85	11.77 ± 0.03	20.87 ± 0.28	34.54 ± 0.36	40.27 ± 0.16	8.42 ± 0.01	18.89 ± 0.10	24.83 ± 0.08	51.39 ± 0.40	9.05 ± 0.01	18.03 ± 0.03	30.16 ± 0.03	46.45 ± 0.14	13.19 ± 0.00	15.07 ± 0.11	26.90 ± 0.05	41.09 ± 0.81
Naringin	16.67 ± 0.79	0.35 ± 0.00	0.52 ± 0.02	0.90 ± 0.00	1.47 ± 0.06	0.38 ± 0.02	1.45 ± 0.01	2.01 ± 0.04	3.50 ± 0.11	0.35 ± 0.03	0.53 ± 0.00	0.78 ± 0.01	1.66 ± 0.08	0.38 ± 0.01	0.65 ± 0.01	1.55 ± 0.01	2.23 ± 0.04
Cinnamic acid	2.43 ± 0.11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Naringenin	7.57 ± 0.12	0.47 ± 0.02	0.50 ± 0.05	0.70 ± 0.01	0.86 ± 0.03	0.42 ± 0.00	0.65 ± 0.01	0.76 ± 0.01	0.83 ± 0.02	0.51 ± 0.03	0.57 ± 0.07	0.83 ± 0.01	1.46 ± 0.04	0.42 ± 0.04	0.59 ± 0.00	0.65 ± 0.02	0.73 ± 0.01
Chlorogenic acid	5.21 ± 0.00	-	0.29 ± 0.07	0.51 ± 0.00	0.57 ± 0.01	0.21 ± 0.04	0.24 ± 0.02	0.35 ± 0.01	0.66 ± 0.03	-	-	0.38 ± 0.04	0.49 ± 0.08	-	0.17 ± 0.04	0.38 ± 0.04	0.57 ± 0.01
Caffeic acid	50.43 ± 0.22	0.13 ± 0.00	0.20 ± 0.00	0.30 ± 0.01	0.35 ± 0.01	0.12 ± 0.01	0.19 ± 0.01	0.28 ± 0.00	0.37 ± 0.02	0.10 ± 0.00	0.25 ± 0.00	0.36 ± 0.02	0.45 ± 0.00	0.11 ± 0.01	0.15 ± 0.01	0.25 ± 0.01	0.35 ± 0.00
p-Coumaric acid	6.18 ± 0.31	0.18 ± 0.01	0.25 ± 0.00	0.31 ± 0.01	0.42 ± 0.00	0.29 ± 0.00	0.30 ± 0.00	0.34 ± 0.00	0.38 ± 0.00	0.12 ± 0.01	0.22 ± 0.01	0.34 ± 0.01	0.42 ± 0.00	0.20 ± 0.00	0.23 ± 0.01	0.32 ± 0.01	0.39 ± 0.01
Ferulic acid	11.85 ± 0.11	0.66 ± 0.00	0.74 ± 0.02	1.25 ± 0.03	1.39 ± 0.02	0.84 ± 0.00	0.97 ± 0.00	1.18 ± 0.03	1.51 ± 0.03	0.41 ± 0.02	0.55 ± 0.00	1.03 ± 0.02	1.43 ± 0.01	0.52 ± 0.03	0.59 ± 0.03	0.77 ± 0.01	1.38 ± 0.06
Sinapinic acid	1.71 ± 0.04	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Total	603.65 ± 1.69	14.90 ± 0.03	25.67 ± 0.50	42.27 ± 0.29	49.94 ± 0.06	12.38 ± 0.09	25.53 ± 0.09	33.77 ± 0.09	65.41 ± 0.38	11.59 ± 0.09	22.01 ± 0.12	37.06 ± 0.10	57.03 ± 0.02	17.00 ± 0.10	20.05 ± 0.10	34.91 ± 0.09	52.97 ± 0.70

Note: [1)]Not detected.

Table 4: The phenolic compounds contents in ethanolic extracts with different mixing ratio of non-glutinous sorghum (*Sorghum bicolor* L. Moench cv. Donganme).

and high-pressure rice cookers, respectively); the figures for mixtures cooked in 10% (v/v) alcohol were 25.34–103.26 and 18.03–108.47 μg/g respectively. Overall, phenolic acid levels were higher in samples cooked in plain water.

The phenolic acids of Donganme sorghums included 4-hydroxybenzoic acid (6.06 μg/g), vanillic acid (2.66 μg/g), rutin (7.98 μg/g), myricetin (8.52 μg/g), quercertin (4.25 μg/g), syringic acid (17.95 μg/g), *t*-3-hydroxy cinnamic acid (454.21 μg/g), naringin (16.67 μg/g), cinnamic acid (2.43 μg/g), naringenin (7.57 μg/g), chlorogenic acid (5.21 μg/g), caffeic acid (50.43 μg/g), *p*-coumaric acid (6.18 μg/g), ferulic acid (11.85 μg/g), and sinapinic acid (1.71 μg/g) (Table 4). The total phenolic acid content of Donganme sorghums was 603.65 μg/g. The predominant phenolic acid was t-3- hydroxycinnamic acid; the levels in mixtures cooked in water were 11.77–40.27 and 8.42–51.39 μg/g (normal and high-pressure rice cookers, respectively); the figures for mixtures cooked in 10% (v/v) alcohol were 9.05–46.45 and 13.19–41.09 μg/g, respectively. The naringin levels of water-cooked samples were 0.35–1.47 and 0.38–3.50 μg/g (normal and high-pressure rice cookers, respectively); the figures for mixtures cooked in 10% (v/v) alcohol were 0.35–1.66 and 0.38–2.23 μg/g, respectively. The syringic acid, caffeic acid, p-coumaric acid and ferulic acid levels of rice/ Donganme sorghum mixtures (5–20% w/w) were 0.37–3.03, 0.11–0.45, 0.12–0.42 and 0.41–1.51 μg/g, respectively. Quercertin, cinnamic acid and sinapinic acid were not detected in cooked rice/ Donganme sorghum mixtures. The total phenolic acid contents in cooked rice/ Donganme sorghum mixtures cooked in water were 14.90–49.94 and 12.38–65.41 μg/g (normal and high-pressure rice cookers, respectively); the figures for mixtures cooked in 10% (v/v) alcohol were 11.59–57.03 and 17.00–52.97 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 [27]. However, Lou et al. [28] found that phenolic levels in hot water extracts of the peel of immature calamondins increased after heating at 150°C for 1.5 hr. Release of naringin, tangeretin, ferulic acid, *p*-coumaric acid, and gallic acid was enhanced. Kim et al. [29] reported that the chlorogenic, ferulic, caffeic, and cinnamic acid contents of small black soybeans increased after heating.

Radical-scavenging activities of cooked rice/sorghum 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 [30]. 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 [31]. The DPPH radical– scavenging activities of cooked rice/sorghum mixtures were compared with that of the standard trolox (Figure 3); activity increased as the sorghum proportion rose. The activity of plain rice ranged from 2.97–5.19 mg TE/100 g by the cooking method. The activities of mixtures of rice/Nampungchal sorghums (20% w/w) cooked in water averaged 36.43 ± 0.64 and 36.80 ± 0.51 mg TE/100 g (normal and high-pressure rice cookers, respectively); the figures for mixtures cooked in 10% (v/v) alcohol were 40.13 ± 0.56 and 40.85 ± 0.72 mg TE 100/g respectively (Figure 3A). The activity of rice/Donganme sorghum mixtures (5–20% w/w) cooked in water were 9.21–27.00 and 11.49–31.63 mg TE/100 g (normal and high-pressure rice cookers, respectively); the figures for mixtures cooked in 10% (v/v) alcohol were 13.50–28.63 and 16.58–31.08 mg TE/100 g, respectively (Figure 3B).

When ABTS is mixed with potassium persulfate in the dark, $ABTS^{+\bullet}$ 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 [32]. We compared the radical-scavenging activities of cooked rice/sorghum mixtures to that of a trolox standard (Figure 4). The radical-scavenging activity increased as the sorghum proportion rose. The activity of plain rice varied by cooking method from 14.07–15.83 mg TE/100 g. The activity of rice/Nampungchal sorghum mixtures (20% w/w) cooked in water in a normal and a high-pressure rice cooker averaged 38.52 ± 0.22 and 41.50 ± 0.28 mg TE/100 g; the figures for mixtures prepared with 10% (v/v) alcohol were 55.18 ± 1.89 and 56.70 ± 2.63 mg TE/100 g, respectively (Figure 4A); and the figures for Donganme sorghums (5–20% w/w) cooked in water and 10% (v/v) alcohol were 15.76–25.95 and 15.27–30.48; and 21.80–58.68 and 23.63–59.65 mg TE/100 g, respectively (Figure 4B). Antioxidants inhibit lipid oxidation, slow aging, and inhibit disease [15]. Recently, it was found that prolonged heat treatment enhanced the antioxidant activities of tomato and coffee [33]. 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 [34]. When rice/sorghum mixes were cooked in normal cookers, radical-scavenging activity was high; such cookers can thus be used for industrial applications.

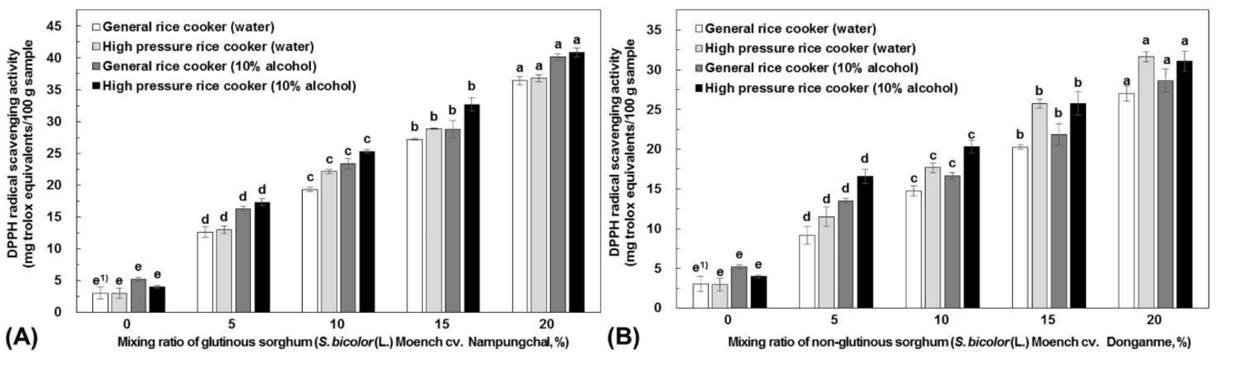

Figure 3: DPPH radical scavenging activity on the ethanolic extracts with different mixing ratio of sorghum (*Sorghum bicolor* L. Moench cv. Nampungchal (glutinous; A), and Donganme (non- glutinous; A)) and cooking methods. [1)]Any means in the same column followed by the same letter are not significantly (p<0.05) different by Duncan's multiple range test.

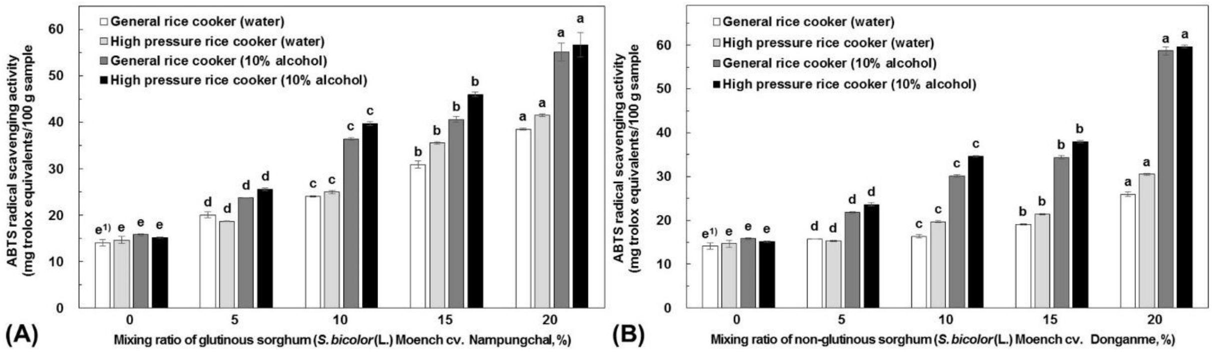

Figure 4: ABTS radical scavenging activity on the ethanolic extracts with different mixing ratio of sorghum (*Sorghum bicolor* L. Moench cv. Nampungchal (glutinous; A), and Donganme (non- glutinous; B)) and cooking methods. [1)]Any means in the same column followed by the same letter are not significantly (p<0.05) different by Duncan's multiple range test.

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