

# Dependence of *Sorghum bicolor* antioxidant activity on harvest time

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**ABSTRACT:** This study was done to examine the correlation between differential contents of antioxidants and their antioxidative activities in the seeds obtained from two sorghum cultivars (*Nampungchal* and *Hwanggeumchal*) according to harvest time post heading, and to understand the properties of the harvested seeds. The seeds were harvested every 5 days from 25–55 days post heading. Length, width, thickness, and morphological properties of the obtained seeds were increased in proportion to the harvest time. The weights per thousand grains, crude fat, and redness ( $a^*$ ) clearly changed depending on the harvest time. The grain weight and fat content showed the highest value at harvest time days 45 (HT 45). The seed coat lightness ( $L^*$ ), crude protein, total polyphenol, flavonoid, and tannin in both cultivars were inversely proportional to the harvest time. In addition, ABTS and DPPH radical scavenging activities were also reduced in inverse proportion to the elapsed harvest time. Antioxidants including polyphenol, flavonoid, and tannin showed positive correlations with the antioxidative activities such as ABTS and DPPH activities, but antioxidants and their activities had no significant correlations between cultivars. We therefore propose that sorghum harvest is performed earlier to improve its antioxidative function.

**KEYWORDS:** polyphenol, seed morphology

## INTRODUCTION

The improvement of life quality and diffusion of a culture of well-being according to economic growth have promoted public interest in functional cereals. Countries where rice is the main food demand functional boiled rice and mixed application of other grains with rice for health promotion. Functional cereals have so far been recognized as crops of a low yield using a small cultivation area, but are preferred by consumers. Since 2007, demand and import of functional cereals in South Korea has been rapidly risen to 69 000 and 50 000 t, respectively<sup>1</sup>. In addition, they are applied in various ways such as new industrial materials, foods, pharmaceuticals, and natural dyes, as well as are employed as excellent crops for conservation of landscape and environment.

Among functional cereals, *Sorghum bicolor* is a representative C<sub>4</sub> crop originated from Africa, which is cultivated mainly in the subtropical and semi-arid regions including India to maintain rainfall below 400 mm per year. *S. bicolor* requires 50% less water

than corn, as it has a high absorption ability and low nutrient demand<sup>2–4</sup>. In addition, it contains a number of bioactive components such as dietary fibre and phenolic compounds, including flavonoid, tannin, and phenolic acids<sup>5–7</sup>.

Phenolic compounds included in sorghum exhibit a strong anti-mutagenic activity and sorghum extracts show a strong antioxidant activity<sup>8–10</sup>. Sorghum functionality has been examined in previous studies such as assays of antioxidant and antibiotic activities after methanol extraction followed by sequential solvent fractionation<sup>11</sup>, measurement of anthocyanin antioxidant activity<sup>12</sup>, and study of phenolic acid, flavonoid, and tannin<sup>13–15</sup>.

Although sorghum has been actively studied for antioxidant components and physiological function, there is no result for changes of major chemical and antioxidant components from the harvested seeds according to cultivation time. Hence, in this study, we examined specific properties of sorghum including measurements of representative antioxidant components and their activities depending on harvest time post heading.

## MATERIALS AND METHODS

### Sorghum seed harvest and treatment

Sorghum cultivars employed in this study (cv. *Nampungchal* and *Hwanfgeumchal*) were cultivated in an experimental field (35° 05' 16'' N, 127° 56' 34'' E) in Sacheon, Gyeongnam, South Korea in 2014. Sorghum was harvested 7 times at intervals of 5 days from 25–55 days post heading (HT 25, 30, 35, 40, 45, 50, 55). The harvested sorghum seeds were well-dried to a moisture content of approximately 13%, and then applied as samples after removing the hull surrounding the grain.

### Morphological properties of seeds

Morphological property of the seeds was examined by 4 repeated experiments to 100 normal grains per individual assay from each harvested sorghum seed. Morphological assay of the seeds was examined for length, width, and thickness. The seed coat colour values such as lightness ( $L^*$ ), redness ( $a^*$ ), and yellowness ( $b^*$ ) were determined by spectrophotometry (Minolta spectrophotometer, cm-3500d, Japan).

### Analyses of chemical composition, total polyphenol, flavonoid, and tannin

The samples were crushed for 10 min at 4600 rpm by a pin mill pulverization (DK201, Sejung Tech, Daegu, Korea) and then a fraction was applied for analysis of chemical compositions according to AOAC method<sup>16</sup>.

Total polyphenol content was assayed by Folin-Ciocalteu phenol reagent<sup>17</sup>. One millilitre of 2%  $\text{Na}_2\text{CO}_3$  solution was mixed with 50  $\mu\text{l}$  of sample; the mixed solution was incubated for 3 min, and then 50  $\mu\text{l}$  of 50% Folin-Ciocalteu reagent (Sigma-Aldrich, St. Louis, MO, USA) was added into the mixed solution. After reacting for 30 min at room temperature, the absorbance at 750 nm of the treated solution was measured (Multiscan GO, Thermo Scientific Co. Ltd., USA). The regression line is represented by  $y = 0.003x$  ( $R^2 = 0.989$ ) in mg of gallic acid (dry basis) per g of sample.

Total flavonoid content was determined according to the method of Dewanto et al<sup>17</sup>. The reaction solution was prepared by 1 ml of distilled water, 75  $\mu\text{l}$  of 5%  $\text{NaNO}_2$  and 250  $\mu\text{l}$  of sample, and then incubated for 5 min at room temperature. The reacted solution was additionally mixed with 500  $\mu\text{l}$  of 1 N NaOH, and then left to react for 11 min at room temperature. The absorbance at 510 nm of the treated solution was measured (Multiscan GO, Thermo Scientific Co. Ltd., USA). The regression

line is represented by  $y = 0.005x$  ( $R^2 = 0.998$ ) in mg of catechin (dry basis) per g of sample.

Total tannin was measured according to the method of Duval and Shetty<sup>18</sup>. Reaction solution was prepared with 1 ml of 95% ethanol, 1 ml of distilled water and 1 ml of sample solution. After mixing, 1 ml of 5%  $\text{Na}_2\text{CO}_3$  solution and 0.5 ml of 1 N Folin-Ciocalteu reagent (Sigma-Aldrich) were added into the mixed solution, and the reaction was allowed to develop colour for 60 min at room temperature. The absorbance at 725 nm of the treated solution was measured (Multiscan GO, Thermo Scientific Co. Ltd., USA). The calibration line ( $y = 0.0097x$ ,  $R^2 = 0.9769$ ) was prepared in ml of tannic acid (dry basis) per g of the sample.

### Assays for ABTS and DPPH radical scavenging activities

The antioxidative activity of the sample was measured by ABTS (2,2'-azino-bis-3-ethylbenzo-thiazoline-6-sulphonic acid, Sigma-Aldrich) and DPPH (1,1-diphenyl-2-picrylhydrazyl, Sigma-Aldrich) radical scavenging activities<sup>19,20</sup>. To measure the ABTS radical scavenging activity, 50  $\mu\text{l}$  of sample was mixed with 1 ml of a diluted ABTS solution, let it react for 30 min at room temperature, and then the absorbance was measured at 735 nm wavelength (Thermo Scientific Co. Ltd., USA). ABTS scavenging activity was expressed in mg of TE (Trolox equivalent antioxidant capacity) per g.

To measure the DPPH radical scavenging activity, 0.2 ml of sample was mixed with 0.8 ml of 0.2 mM DPPH solution dissolved in 99.9% methanol, let react for 30 min, and the absorbance at 520 nm was measured (Thermo Scientific Co. Ltd., USA). DPPH scavenging activity was expressed in mg of TE per g.

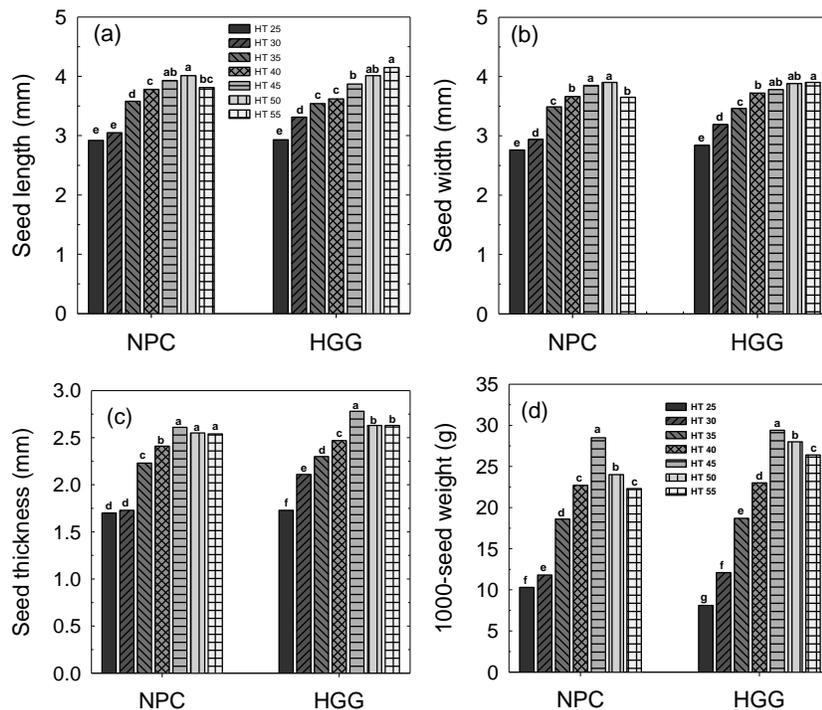
### Statistical analysis

The average value of collected data was compared, with a significance level of 5%, through Duncan's multiple range test (DMRT) by PROC ANOVA procedures of SAS 9.2 (Cary, NC).

## RESULTS AND DISCUSSION

### Morphological properties of sorghum seeds

To analyse the seed morphological properties at each harvest time, the harvested sorghum seeds were examined for length, width, thickness, and weight per thousand grains (Fig. 1). As a result of seed length, *Nampungchal* seed (NPC; 3.58 mm) was 0.52 mm longer than that of *Hwanfgeumchal*



**Fig. 1** Morphological properties according to each harvest time of sorghum; (a) seed length, (b) seed width, (c) seed thickness, (d) thousand-grain weight. The different superscripts in bar graphs indicate significant differences at  $p < 0.05$  by Duncan's multiple range test.

seed (HGC; 3.06 mm). The seed length showed a clear difference depending on the harvest time. The longest lengths of NPC and HGC at HT 50 and HT 55 were 4.01 mm and 4.15 mm, respectively, whereas shortest lengths of both seeds occurred at the earliest harvest time of HT 25 as 2.92 and 2.93 mm, respectively. NPC and HGC showed the longest width of 3.90 mm at HT 50 and HT 55, respectively. NPC and HGC showed the maximum thickness of 2.54 mm and 2.78 mm at HT 45.

Length and width of the college glutinous corn increase according to harvest time<sup>21</sup>. Hence it is assumed that sorghum has differential growth properties and harvest time with college glutinous corn. Weight per thousand grains showed a clear difference according to harvest time. When the seeds of NPC and HGC presented the longest width and the maximum thickness, they showed the maximum weights of 28.5 g and 28.4 g, respectively, at HT 45. Both cultivars showed the minimum weights at the earliest harvest time of HT 25. Hence, according to width, thickness, and weight of the seeds, we suggest that sorghum seed is optimal at HT 45.

Lightness ( $L^*$ ) and yellowness ( $b^*$ ) among seed coat colours was inversely proportional to the har-

vest time, whereas redness ( $a^*$ ) followed an opposite trend (Table 1). Interestingly however, the  $b^*$  value of HGC increased depending on the harvest time. It is assumed that the differential results between cultivars are greatly influenced by genetic differences. On the other hand, as shown in other crops<sup>22,23</sup>, the value of  $a^*$ , highly related to the content of anthocyanin in sorghum, increases at later harvest time. Although more detailed research remains to be done in the future, we suggest that the harvest time is considered for utilization of anthocyanins.

### Chemical compositions

As shown in Table 2, chemical compositions including protein, fat, and ash showed similar values to those of Korean sorghum<sup>24</sup>. The content of crude protein in both cultivars decreased as the cultivation period elapsed. The content of crude protein (8.58–10%) in NPC was slightly lower than that of HGC (9.09–14%). Crude fat in NPC showed the lowest amount (3%) at an earliest harvest time (HT 25), where it showed the largest amount of 5%. Crude fat in HGC exhibited also the highest amount (5%) at HT 45. In contrast to the content of crude

**Table 1** Changes of seed coat colour according to harvest time of sorghum.

HT (day)	NPC			HGC		
	<i>L</i> *	<i>a</i> *	<i>b</i> *	<i>L</i> *	<i>a</i> *	<i>b</i> *
25	85.6 ± 2.7 <sup>a</sup>	3.0 ± 1.5 <sup>d</sup>	58.9 ± 2.1 <sup>a</sup>	86.0 ± 2.1 <sup>a</sup>	3.6 ± 1.2 <sup>e</sup>	47.1 ± 2.3 <sup>b</sup>
30	84.3 ± 4.2 <sup>a</sup>	3.0 ± 1.7 <sup>d</sup>	59.0 ± 4.2 <sup>a</sup>	84.7 ± 2.5 <sup>ab</sup>	4.0 ± 1.7 <sup>e</sup>	47.0 ± 8.7 <sup>b</sup>
35	72.3 ± 5.5 <sup>b</sup>	17.7 ± 2.9 <sup>c</sup>	56.3 ± 2.9 <sup>ab</sup>	83.7 ± 3.5 <sup>ab</sup>	7.3 ± 3.1 <sup>d</sup>	48.3 ± 7.6 <sup>b</sup>
40	71.3 ± 7.1 <sup>b</sup>	20.0 ± 7.5 <sup>bc</sup>	55.0 ± 3.6 <sup>ab</sup>	74.3 ± 5.0 <sup>b</sup>	15.0 ± 5.2 <sup>c</sup>	50.7 ± 8.1 <sup>b</sup>
45	70.7 ± 9.1 <sup>b</sup>	17.7 ± 8.0 <sup>c</sup>	53.7 ± 3.8 <sup>b</sup>	58.3 ± 2.5 <sup>c</sup>	30.3 ± 3.2 <sup>b</sup>	53.0 ± 2.0 <sup>ab</sup>
50	55.3 ± 3.2 <sup>c</sup>	27.7 ± 3.5 <sup>b</sup>	50.7 ± 4.2 <sup>b</sup>	55.3 ± 9.0 <sup>c</sup>	37.0 ± 6.1 <sup>ab</sup>	56.7 ± 4.0 <sup>a</sup>
55	51.3 ± 1.1 <sup>c</sup>	40.0 ± 3.6 <sup>a</sup>	46.7 ± 2.1 <sup>c</sup>	41.3 ± 2.5 <sup>d</sup>	39.3 ± 0.6 <sup>a</sup>	52.3 ± 2.3 <sup>b</sup>

*L*\*: lightness; *a*\*: redness (+ red, – green); *b*\*: yellowness (+ yellow, – blue). Values are mean ± SD. Different superscript letters in the same column indicate significant difference based on Duncan's multiple range tests ( $p < 0.05$ ).

**Table 2** Chemical compositions according to harvest time of sorghum seed.

Cultivar	HT (day)	Crude protein (%)	Crude fat (%)	Crude ash (%)	K (%)	Ca (%)	Mg (%)	Na (%)
NPC	25	10.35	3.22	1.20	0.632	0.039	0.280	0.008
	30	10.03	3.69	1.20	0.674	0.043	0.333	0.004
	35	9.72	3.80	1.30	0.555	0.023	0.231	0.004
	40	9.65	4.09	1.33	0.545	0.024	0.289	0.005
	45	9.28	4.59	1.35	0.501	0.021	0.270	0.007
	50	9.28	4.26	1.41	0.462	0.023	0.246	0.007
	55	8.58	4.19	1.53	0.456	0.025	0.238	0.007
HGC	25	13.82	2.25	1.10	0.704	0.080	0.376	0.006
	30	10.47	3.19	1.11	0.630	0.045	0.310	0.005
	35	10.10	3.85	1.11	0.560	0.032	0.279	0.006
	40	9.53	3.98	1.12	0.530	0.025	0.267	0.006
	45	9.28	4.76	1.13	0.520	0.021	0.214	0.005
	50	9.09	4.44	1.13	0.559	0.021	0.332	0.007
	55	9.09	4.33	1.18	0.502	0.020	0.269	0.005

protein as described above, the content of ash in both cultivars increased as the harvest time elapsed. The contents of ash in NPC and HGC increased from 1.20–2% and from 1.10–1%, respectively.

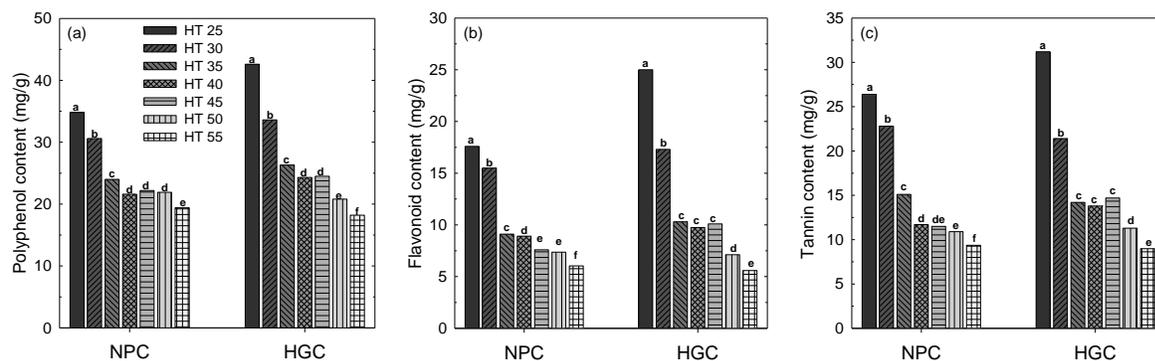
The contents of magnesium and sodium in both cultivars changed from 0.214–0.376% and from 0.004–0.008%, respectively, both of which did not show any apparent difference with harvest time. However, the content of potassium in NPC and HGC showed the highest values of 0.674% and 0.704% at an earlier harvest time (HT 30 and HT 25, respectively), which decreased depending on the elapsed harvest time. Hence the content of potassium in NPC and HGC at HT 55 showed the lowest values of 0.456% and 0.502%, respectively. After the content of calcium decreased from HT 25 to HT 35, it remained at a constant level.

Taken together, crude protein and ash contents in both cultivars increased according to the elapsed

harvest time, whereas fat, potassium, and calcium contents showed the opposite trend. Hence we suggest that sorghum seed is harvested at an early time to obtain the maximal nutrient composition.

#### Antioxidant components

Phenolic hydroxyl radicals have been known to play physiological roles such as antioxidants, anticancer, and antibiotics through combination with a macromolecule including proteins<sup>25</sup>. Polyphenolic compounds among antioxidant substances contained in crops exhibit excellent antioxidant properties owing to the phenolic ring that stabilizes free radicals<sup>26,27</sup>. Both sorghum cultivars maintained higher total polyphenolic content at an earlier harvest time (Fig. 2a). NPC showed differences in total polyphenol content between the earliest harvest time (34.8 mg/g at HT 25) and the latest harvest time (15.4 mg/g at HT 55), but relatively



**Fig. 2** Changes of antioxidants according to each harvest time of sorghum; (a) polyphenolic content, (b) flavonoid content, (c) tannin content. Different superscripts in bar graphs indicate significant differences at  $p < 0.05$  by Duncan's multiple range test.

small differences in thousand-grain weight (21.6–22.2 mg/g) among HT 40, 45, and 50. The content of average polyphenol in HGC maintained a higher value of 2.3 mg/g or more than that of NPC. HGC presented differences in the polyphenol content of 24.4 mg/g between the earliest harvest time (HT 25) and the latest harvest time (HT 55). This change of HGC was 5 mg/g higher than that of NPC.

Flavonoid compounds mainly include anthocyanidins, flavonols, flavones, catechins, and flavanones. A specific flavonoid compound has been known to exhibit various physiological activities including antioxidant and antibiotic depending on the structure<sup>28</sup>. Flavonoids showed the highest amount at HT 25 as 17.6 and 25.0 mg/g in NPC and HGC, respectively (Fig. 2b).

Tannin, which has a close correlation with taste, flavour, and colour of tea, inhibits microbial growth and enzymatic activity<sup>29,30</sup>. Tannin was also decreased by the elapsed harvest time. The content at an early harvest time of HT 25 was 26.4 and 31.2 mg/g in NPC and HGC, respectively. The amounts correspond to a 2.8 and 3.5 folds higher than those at the latest harvest time of HT 55 for NPC and HGC, respectively (Fig. 2c).

The contents of total polyphenol, flavonoid, and tannin in an ethanol extract of sorghum are 6.23–7.51, 1.76–2.25, and 3.71–4.34 mg/g, respectively<sup>31</sup>. The previous study showed higher values than those of current study. It is assumed that these differences are owing to the extracted solvents, year of production, or grown environment. In addition, our results show that the content of antioxidant components varies clearly depending on harvest time of sorghum. Hence we suggest that sorghum harvest time be determined depending on

the purpose of sorghum application.

#### Antioxidant activity

Bioactive substances present an antioxidant activity by inhibition of major substances in foods such as fat via donating electrons to active radicals. Bioactive substances are thought to inhibit ageing via scavenging of active radicals in body<sup>32</sup>. Action of radical scavenging plays a critical role to prevent body from diseases and ageing<sup>28</sup>.

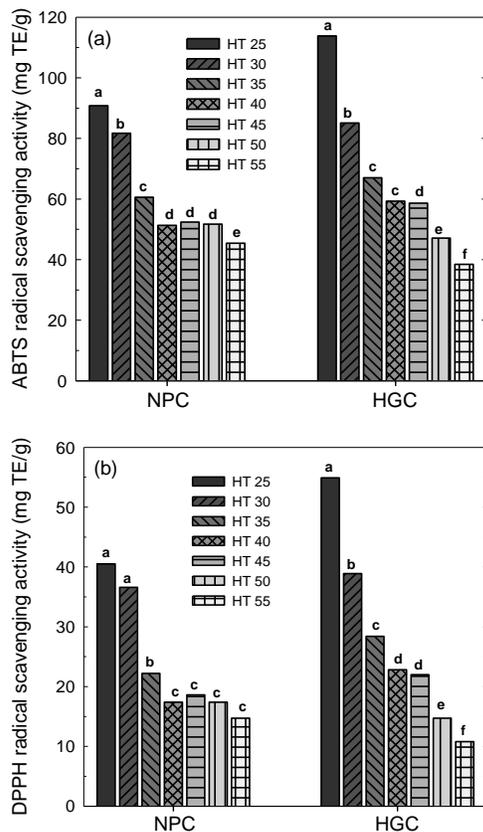
ABTS radical scavenging activity of sorghum at each harvest time showed the highest values at HT 25 for both NPC (90.8 mg/g TE) and HGC (113.8 mg/g TE) (Fig. 3). The activity clearly decreased depending on the elapsed harvest time, which was presented by 45.4 and 38.4 mg/g TE for NPC and HGC at HT 55, respectively. DPPH radical scavenging activity also showed the highest values at an earliest harvest time of HT 25 for both NPC (40.5 mg/g TE) and HGC (54.9 mg/g TE).

Antioxidant activity depends on harvest time due to the high correlations between phenolic compounds and ABTS or DPPH radical scavenging activity<sup>33</sup>. As shown in foxtail millet and proso millet<sup>33</sup>, it was expected that the antioxidant components and their activities maintain higher values at long cultivation periods of the later harvest time. However, in this study, since a high activity was observed at an early harvest time, a more detailed study is required to determine whether the antioxidant components and their activities result from differences in composition or from differential content ratio due to weight gain. Otherwise, the bran layer of sorghum exhibits higher activities for ABTS radical (66.4 mg/g TE) and DPPH radical (35.6 mg/g TE)<sup>34</sup>. Since these results are very important for the

**Table 3** Correlations between antioxidants, antioxidation, cultivar and harvest time of sorghum seed.

	Polyphenol	Flavonoid	Tannin	ABTS	DPPH	Cultivar
Flavonoid	0.990**					
Tannin	0.976**	0.977**				
ABTS	0.988**	0.982**	0.974**			
DPPH	0.983**	0.978**	0.964**	0.993**		
Cultivar	0.167 <sup>ns</sup>	0.197 <sup>ns</sup>	0.084 <sup>ns</sup>	0.124 <sup>ns</sup>	0.140 <sup>ns</sup>	
HT	-0.865**	-0.843**	-0.872**	-0.884**	-0.888**	0.000

ns = no significance; \*\* = significance at  $p < 0.01$ .



**Fig. 3** Changes of antioxidant activities according to each harvest time of sorghum; (a) ABTS radical scavenging activity, (b) DPPH radical scavenging activity. Different superscripts in bar graphs indicate significant differences at  $p < 0.05$  by Duncan's multiple range test.

utilization of sorghum, we suggest that more studies of the bioactive substances of sorghum should be carried out in the future.

The components of antioxidant showed significantly positive correlations among them such as polyphenol, flavonoids, and tannin (Table 3). ABTS or DPPH radical scavenging activity showed also a significant correlation with the antioxidant compo-

nents. In addition, ABTS radical scavenging activity exhibited a significant correlation with the DPPH radical scavenging activity. However, cultivar types showed no significant correlation with antioxidant components, antioxidation, or harvest time. In addition, harvest time showed significantly negative correlation with antioxidant components or antioxidation. We therefore suggest that sorghum with excellent antioxidant power should be applied as a good material for the production of functional foods at a suitable harvest time.

## CONCLUSIONS

In this study, we examined general characteristics as well as representative antioxidant components and their activities of sorghum seeds at various harvest times. The morphological properties and chemical compositions showed various properties depending on the harvest time. Total polyphenols, flavonoids, and tannin contents, which are representative antioxidant components of the sorghum seeds, were in inversely proportional to harvest time. The earlier harvest time showed more ABTS and DPPH radical scavenging activities. These results reveal a high correlation between antioxidant components and ABTS or DPPH radical scavenging activities. Harvest time showed high negative correlations with antioxidant components or their activities. We therefore suggest that during the cultivation and production process of sorghum, it is critically important to select the cultivar depending on the purpose of application.

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