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Fluctuations in Phenolic Content and Antioxidant Capacity of Green Vegetable Juices during Refrigerated Storage

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ABSTRACT: Shinseoncho and kale were made into green vegetable juices by building block [shinsenocho branch (SB), shinsenocho leaf (SL), kale branch (KB), and kale leaf (KL)]. Fluctuations in their phenolic contents and antioxidant capacities were analyzed during refrigerated storage at 4°C for 28 days. Total polyphenolic contents of leaf parts showed a decreasing tendency after 4 days (SL) or 7 days (KL), whereas branch parts showed fluctuating values during the entire storage period. The 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical scavenging capacity was rapidly decreased in SB and in SL at 28 days ($P<0.001$), whereas KL showed a slightly increasing tendency after 14 days. For the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity, SL showed a sharp fall at 28 days ($P<0.001$), and KL showed a decreasing tendency after 14 days ($P<0.001$). SB showed a steady decrease during the entire storage period and KB indicated a nearly zero (0.97%) at 28 days. Pearson's coefficients for the correlation between antioxidant capacities measured by the ABTS and DPPH assays, and the total polyphenolic contents were determined. The results showed that the ABTS assay (r=0.934, $P<0.001$) was more strongly positively correlated with the total phenolic contents than the DPPH assay (r=0.630, $P<0.001$). In conclusion, when considering all building blocks, green vegetable juices, including kale and shinseoncho may have kept antioxidant capacities for up to 14 days under refrigeration, and the ABTS assay better reflects a positive correlation with the total phenolic contents when compared to the DPPH assay.

Keywords: green vegetable juice, building block, refrigerated storage, antioxidant capacity, phenolic content

INTRODUCTION

The importance of dietary antioxidant components for the prevention of some diseases, including cancer and heart disease, has attracted much attention during the last decade. Epidemiological studies have shown that many phytonutrients from fruits and vegetables may be beneficial in protecting the human body against damage by reactive oxygen and nitrogen species (1,2).

Recent studies have begun to demonstrate that a number of vegetable juices display high total antioxidant capacities when quantified using biochemical assays (3,4). However, antioxidant capacities of only some of the vegetable juices from tomatoes and carrots have been studied, and research regarding green vegetable juices has not been elucidated (5-7).

Green vegetable juices can be conveniently used to meet the recommendations of daily vegetables. Especially, kale and shinseoncho juices are two kinds of popular green vegetable juices consumed in Korea, which are known as rich sources of vitamins, flavonoids, and minerals (8). In spite of the popularity of kale and shinseoncho juices in Korea, little is known about their potential effects on human health. Moreover, some researchers have studied the prevention effects of whole green juices on cancer and cardiovascular diseases. Although the antioxidant capacities of vegetable juices have been reported little information is available on the loss of antioxidants due to processing and cold storage (9-11).

Therefore, the current study aims to determine the antioxidant capacities and the total phenolic contents of green vegetable juices by building block (branch and leaf parts) of kale and shinseoncho as well as their changes of the antioxidant capacities during the refrigerated storage period. Pearson's correlation coefficients were calculated between the antioxidant capacities determined by the ABTS and DPPH assays, and the total phenolic contents.

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MATERIALS AND METHODS

Preparation of green vegetable juices by building block

Shinseoncho (*Angelica utilis makino*) and kale (*Brassica oleraceae* L. var. *acephala* DC.) were purchased from a local organic market (Suwon, Korea) in July, 2012. The raw vegetables were washed with tap water twice and then air dried at room temperature for 3 h. Kale and shinseoncho were divided into branch [shinsenocho branch (SB) and kale branch (KB)] and leaf parts [shinsenocho leaf (SL) and kale leaf (KL)] by naked eye identification. The samples were ground with an electronic grinder (Hanil Electronics Corp., Wonju, Korea) after adding distilled water three times and then filtrated with four-fold gauze. The sample of 10 mL by the building block was contained into a 15 mL screw capped conical tube. The samples were stored in a refrigerator at 4°C until use. The tubes were centrifuged at 150 *g* for 10 min prior to analysis for the fluctuation of the phenolic contents and antioxidant capacities.

Total polyphenolic content

The total polyphenolic content was determined using the Folin-Ciocalteu method (12) with some modifications. The total polyphenolic concentration was calculated from a calibration curve using gallic acid as a standard. Briefly, 0.79 mL of distilled water, 0.01 mL of sample, and 0.05 mL of Folin-Ciocalteu reagent were added to a 1.5-mL eppendorf tube and then mixed. After exactly 1 min, 0.15 mL of sodium carbonate solution (20 g/100 mL) was added, and the mixture was then mixed. After incubation for 120 min at room temperature, the absorbance was read at 750 nm.

Antioxidant capacity

The 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical scavenging capacity was measured according to the method described by Re et al. (13), with some modifications. The ABTS radical was generated by adding 7 mmol/L ABTS to a 2.45 mmol/L potassium persulfate solution and then allowing the mixture to stand overnight in a dark at room temperature. The ABTS radical solution was then diluted with distilled water to obtain an absorbance of 1.4~1.5 at 414 nm. Next, 1 mL diluted ABTS radical solution was added to 50 μL sample. After incubation for 60 min at room temperature, the absorbance was measured at 414 nm. The ABTS radical scavenging capacity was calculated using the following equation:

$$\text{ABTS radical scavenging capacity (\%)} = \frac{1 - A_s}{A_c} \times 100$$

where A_s is the absorbance in the presence of sample, and A_c is the absorbance in the absence of sample.

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity was measured according to the method described by Cheung et al. (14), with some modifications. Briefly, an aliquot of 0.8 mL of 0.2 mmol/L DPPH ethanolic solution was mixed with 0.2 mL of sample. The mixture was then vigorously shaken and incubated under subdued light for 10 min. The absorbance was measured at 520 nm. The DPPH radical scavenging capacity was calculated using the following equation:

$$\text{DPPH radical scavenging capacity (\%)} = \frac{1 - A_s}{A_c} \times 100$$

where A_s is the absorbance in the presence of sample, and A_c is the absorbance in the absence of sample, respectively.

Statistical analysis

All statistical analyses were conducted using the Statistical Package for Social Sciences, version 12.0 (SPSS Inc., Chicago, IL, USA). The data were reported as the means ±standard deviations of triplicate determinations and coefficient of variation. The differences among samples were evaluated statistically by one-way analysis of variance and Duncan's multiple tests. Pearson's correlation coefficients were calculated in order to characterize the relationship between antioxidant capacities determined by storage period, the ABTS and DPPH assays, and the total polyphenolic contents. All tests were performed at a statistical significance level of $P<0.05$, $P<0.01$, and $P<0.001$.

RESULTS AND DISCUSSION

Fluctuations in the total polyphenolic contents of green vegetable juices during refrigerated storage

Vegetable juices are a good source of biological active antioxidant compounds, which may provide protection against the development of a number of diseases (15). Phenolics have high-levels of antioxidants known as aromatic secondary plant metabolites because of their ability to scavenge free radicals and active oxygen species such as singlet, superoxide free radicals and hydroxyl radicals (16). Polyphenolic compounds are also thought to be particularly important in the pathologies of heart disease, hypertension and age-related degeneration (17-19). Therefore, nutritional quality of vegetable juices has been primarily related to their richness in polyphenols (20).

Total polyphenolic contents are commonly quantified using the Folin-Ciocalteu method (21). Several studies have indicated a change in the phenolic compound pro-

file of different fruits and vegetables upon chilled storage (i.e., 4°C), (22-24). In this study, changes of the total polyphenolic contents of green vegetable juices by building block with the increase of time at refrigerated storage (4°C) were investigated using the Folin-Ciocalteu method (Table 1). During the storage period, the total polyphenolic content of SB showed fluctuating values ranging from 70.25 to 80.83 μg/mL at 14 days, and after that, it was increased (91.05~92.82 μg/mL) at 21 and 28 days ($P<0.001$), whereas KB indicated fluctuating values (101.98~110.44 μg/mL) during the entire storage period ($P<0.05$). SL showed a maximum value (436.89 μg/mL) at 2 days, and after that, it indicated a decreasing tendency ($P<0.001$). KL showed an increasing tendency ranging from 231.71 to 232.42 μg/mL at 24 h and 4 days of storage period, and after that, it indicated a decreasing tendency at 28 days (187.29 μg/mL, $P<0.001$). From these results, the total polyphenolic contents of leaf parts indicated a decreasing tendency after 4 days (SL) or 7 days (KL) during refrigerated storage, whereas branch parts generally showed fluctuating values during the entire storage period. SB and KB showed maximum values (SB: 92.82 μg/mL, KB: 110.44 μg/mL) at 21 days. Total polyphenolic contents of leaf parts were more likely to be affected than branch parts under the refrigeration storage.

Some studies have reported that total phenolic content in plant materials showed fluctuating values during refrigerated storage at 4°C. Kevers et al. (22) observed an increase in the phenolic contents of leek and asparagus in the first days of refrigerated storage. And Piljac-Žegarac et al. (25) reported the fluctuations of total phenolic content of six fruit juices (cranberry, black currant, blueberry, pomegranate, cherry, and strawberry) under refrigeration at 4°C for 29 days. These previous reports are in agreement with the present results.

Hertog et al. (26) reported that kale is a leafy vegetable rich in health-promoting flavonols, and it has high concentrations of the flavonol aglycones, kaempferol, and quercetin (27). Structurally different polyphenol compounds, including flavonol glycosides are formed by various glycosylation and acylation processes, known to be affected by temperature and radiation (28). Some researchers also reported that flavonoids, including flavonols could be influenced by temperature. Low temperature enhanced phenolic compounds and total flavonoids as a result of enzymatic repair inhibition, which combined with higher quantities of reactive oxygen species (ROS) (29,30). According to Pinelo et al. (31), an increase in antioxidant capacity may be explained by the strong tendency of polyphenols to undergo polymerization reactions, whereby the resulting oligomers possess larger areas available for charge delocalization. When the degree of polymerization exceeds a critical value, the increased molecular complexity and steric hindrance reduce the availability of hydroxyl groups, which results in a decrease in antiradical capacity.

Moreover, the Folin-Ciocalteu assay is one of the oldest and widely accepted method used to evaluate the total phenolic content in vegetables (32,33). However, this method presents the major disadvantage of overestimating the polyphenol content, since the Folin-Ciocalteu reagent reacts with many reducing substances such as vitamin C, sugar, and amino acids (34). Thus, we assumed that fluctuating values of the total polyphenolic contents in green vegetable juices by building block during the refrigerated storage period in this study was observed due to decreasing temperature and the specificity of the assay.

The effect of refrigerated storage on antioxidant capacities of green vegetable juices

The changes of the ABTS and DPPH radical scavenging capacities of green vegetable juices by building block during refrigerated storage are shown in Table 2 and Table 3, respectively. At the initial time, the ABTS radical scavenging capacity showed the highest value in SL (66.09%), whereas the DPPH radical scavenging capacity was observed at KL (84.83%). Branch parts generally indicated relatively lower values when compared to leaf parts in both, ABTS (SB: 7.70%, KB: 9.61%) and DPPH (SB: 35.44%, KB: 52.89%) radical scavenging capacities.

Table 1. Fluctuations in the total polyphenolic contents of green vegetable juices by building block during refrigerated storage (μg/mL)

Sample[1)]	Time (day)							
	0	1	2	4	7	14	21	28
SB***	75.89±3.40^{bc}	71.31±3.71^{bc}	78.01±2.20^{bc}	80.83±2.66^{b}	70.25±1.62^{c}	76.60±1.22b^{bc}	92.82±2.20^{a}	91.05±3.81^{a}
SL***	423.14±21.23^{ab}	407.28±28.40^{abc}	436.89±2.80^{a}	397.40±2.44^{abc}	370.96±3.71^{cd}	335.01±14.36^{d}	375.90±0.61^{bcd}	346.99±6.94^{d}
KB*	107.27±4.27^{ab}	104.10±4.77^{b}	105.51±3.40^{ab}	107.97±1.83^{ab}	102.69±1.83^{b}	103.74±2.12^{b}	110.44±2.66^{a}	101.98±4.00^{b}
KL***	218.49±12.16^{ab}	232.42±3.71^{a}	232.42±1.22^{a}	231.71±8.66^{a}	202.81±2.20^{bc}	205.27±2.80^{bc}	206.33±5.50^{bc}	187.29±1.06^{c}

Values are expressed as the means±SD of three replicate tests.
Significantly different at *$P<0.05$ and ***$P<0.001$.
Different letters (a-d) mean significantly different in a row.
[1)]SB, shinsenocho branch; SL, shinsenocho leaf; KB, kale branch; KL, kale leaf.

Table 2. Fluctuations in the ABTS radical scavenging capacities of green vegetable juices by building block during refrigerated storage (%)

Sample[1)]	Time (day)							
	0	1	2	4	7	14	21	28
SB	7.70±0.87^{c}	10.40±0.23^{b}	14.13±1.41^{a}	12.19±0.90^{ab}	14.39±0.82^{a}	14.59±0.43^{a}	10.84±0.45^{b}	4.78±0.09^{d}
SL	66.09±1.43^{a}	54.79±0.42^{bc}	55.24±0.52^{bc}	61.48±0.25^{a}	56.05±1.16^{b}	53.15±0.49^{c}	55.80±0.89^{bc}	39.10±0.57^{d}
KB	9.61±0.82^{b}	10.82±0.13^{ab}	13.88±0.86^{a}	11.04±1.54^{ab}	11.83±1.41^{ab}	14.02±0.73^{a}	12.67±0.32^{ab}	10.87±0.17^{ab}
KL	38.51±1.08^{cd}	35.48±0.50^{d}	37.91±1.15^{cd}	38.65±1.87^{cd}	35.67±1.12^{d}	44.05±0.60^{b}	52.16±1.62^{a}	42.18±2.01^{bc}

Values are expressed as the means±SD of three replicate tests.
Significantly different at $P<0.001$.
Different letters (a-d) mean significantly different in a row.
[1)]SB, shinsenocho branch; SL, shinsenocho leaf; KB, kale branch; KL, kale leaf.

Table 3. Fluctuations in the DPPH radical scavenging capacities of green vegetable juices by building block during refrigerated storage (%)

Sample[1)]	Time (day)							
	0	1	2	4	7	14	21	28
SB	35.44±5.14^{a}	31.23±4.52^{ab}	28.70±1.64^{abc}	24.70±1.78^{bc}	26.75±1.17^{abc}	23.45±2.29^{bc}	25.09±1.86^{abc}	19.74±2.39^{c}
SL	63.47±14.05^{a}	76.48±6.27^{a}	72.74±0.72^{a}	78.69±1.20^{a}	82.67±0.00^{a}	73.49±4.03^{a}	71.33±1.72^{a}	29.50±9.77^{b}
KB	52.89±5.91^{ab}	51.32±0.73^{ab}	57.41±1.21^{a}	47.61±2.76^{ab}	46.08±0.33^{ab}	42.33±3.39^{b}	27.85±5.82^{c}	0.97±0.22^{d}
KL	84.83±1.55^{a}	79.75±0.99^{a}	82.17±2.73^{a}	85.14±0.44^{a}	83.53±1.85^{a}	56.03±1.20^{b}	18.80±7.87^{c}	15.36±3.48^{c}

Values are expressed as the means±SD of three replicate tests.
Significantly different at $P<0.001$.
Different letters (a-d) mean significantly different in a row.
[1)]SB, shinsenocho branch; SL, shinsenocho leaf; KB, kale branch; KL, kale leaf.

The DPPH radical scavenging capacity generally showed a higher value than the ABTS radical scavenging capacity in green vegetable juices by building block. It has been previously reported that antioxidant capacities determined by *in vitro* assays, including the ABTS and DPPH assays, differ when applied to food analysis (35). The DPPH method combines reactions of electron transfer with hydrogen atom transfer while the ABTS method only determines a single electron transfer reaction (36).

The changes of ABTS radical scavenging capacity during the storage period was rapidly decreased in SB and SL at 28 days ($P<0.001$), whereas KB indicated fluctuating values during the entire storage period, and KL showed a slightly increasing tendency after 14 days. For the DPPH radical scavenging capacity, SB and SL indicated significant declines at 28 days ($P<0.001$), and KB started to decrease after 14 days followed by a sharp fall after 21 days ($P<0.001$). KL also showed a decreasing tendency after 14 days ($P<0.001$). Especially, the DPPH radical scavenging capacity of KB indicated a nearly zero (0.97%) at 28 days, and KL also showed a very low level at 21 (18.80%) and 28 days (15.36%). From these results, the DPPH radical scavenging capacity was generally more affected than the ABTS radical scavenging capacity by increasing the refrigerated storage time. The DPPH radical scavenging capacity of kale decreased more rapidly than that of shinseoncho, whereas the ABTS radical scavenging capacity of kale was less affected than that of shinseoncho by refrigerated storage.

Correlation between antioxidant capacities measured by the ABTS and DPPH assays, and the total polyphenolic contents

The antioxidant capacity as well as the total phenolic content are associated with the hydroxyl groups of a molecule, so they depend on the phenolic compound chemical structure, namely the number and arrangement of the hydroxylated groups (37,38). Gorinstein et al. (39) reported a high correlation between polyphenols content in several fruits and antioxidant capacities measured by the ABTS and DPPH assays. Dudonné et al. (40) reported a strong positive correlation between ABTS and DPPH assays with a Pearson's correlation coefficient of r=0.906 in 30 aqueous plant extracts.

Therefore, in this study, Pearson's coefficients for the correlation between antioxidant capacities measured by the ABTS and DPPH assays, and the total polyphenolic content using the Folin-Ciocalteu method were determined (Table 4). The results showed that the antioxidant capacity by the ABTS assay (r=0.934, $P<0.001$) was more strongly positively correlated with the total polyphenolic content when compared to the DPPH assay (r=0.630, $P<0.001$). These results suggest that the ABTS assay better reflects a higher positive correlation between antioxidant capacity and the total phenolic content than the DPPH assay.

Table 4. Pearson's coefficients for the correlation between antioxidant capacities measured by the ABTS and DPPH assays, and the total polyphenol contents using the Folin-Ciocalteu method

Variables	Phenolics	ABTS	DPPH
Phenolics	1		
ABTS	0.934[1)]	1	
DPPH	0.630	0.596	1

[1)]Pearson's coefficient by Pearson's correlation.
The correlation is significantly different at $P<0.001$.

Some studies reported that the correlation between the total polyphenolic content and the ABTS assay was stronger than the DPPH assay (40,41), which is in agreement with the present study. A previous study showed that the antioxidant capacity of a sample depends on the solvent used. The ABTS and DPPH assays are *in vitro* models, which do not assess all of the antioxidant capacities in foods (42). Some researchers (43) reported that the reason for a difference in the correlation coefficients is as follows: The ABTS assay is based on the generation of a blue/green $ABTS^{\cdot+}$, which is applicable to both hydrophilic and lipophilic antioxidant systems, whereas the DPPH assay uses a radical dissolved in organic media, therefore, it is applicable to hydrophobic systems. In this study, for the preparation of green vegetable juices by building block, water was used. Thus, fat soluble antioxidants may have not been extracted to the full extent in green vegetable juices by building block. Floegel et al. (41) also reported that for the extraction of antioxidants, methanol was used for antioxidant capacity. The fat soluble antioxidants may have not been extracted to the full amount. Nevertheless, the data showed that the antioxidant capacities measured by the ABTS assay were strongly correlated with the phenolic contents of the 50 most popular antioxidant-rich foods of the US diet. Thus, they concluded that the ABTS assay better reflects the antioxidant capacities of a variety of foods containing hydrophilic, lipophilic, and highly pigmented antioxidant compounds than the DPPH assay. The findings of the present study showed that the ABTS assay correlated more with the total polyphenolic contents than the DPPH assay when applied to green vegetable juices, including kale and shinseoncho.

Pearson's coefficients for the correlation between antioxidant capacities measured by the ABTS and DPPH assays, and the total polyphenolic contents of green vegetable juices by building block during refrigerated storage were also determined, which are shown in Table 5. Total polyphenolic contents of SL ($r=-0.720$, $P<0.001$) and KL ($r=-0.806$, $P<0.001$) showed a negative correlation with an increase of storage time, whereas SB showed a positive correlation ($r=0.767$, $P<0.001$). The DPPH assay showed a negative correlation ($P<0.001$) with elevated storage time in all of the samples, whereas the ABTS assay showed a negative correlation ($r=-0.739$, $P<0.001$) in SL, but KL showed a positive correlation ($r=0.694$, $P<0.001$). The most positive correlation between the ABTS and DPPH assays, and the total polyphenolic content by building block was observed in SL (ABTS: $r=0.901$, DPPH: $r=0.749$, $P<0.001$). Floegel et al. (41) also reported the different correlation coefficients between antioxidant capacities detected by the ABTS and DPPH assays, and the phenolic contents of fruits, vegetables, and beverages. The present study showed different correlation coefficients in green vegetable juices by building block, including shinseoncho and kale, which was in agreement with the previous study.

Table 5. Pearson's coefficients for the correlation between antioxidant capacities measured by the ABTS and DPPH assays, and the total polyphenolic contents of green vegetable juices by building block using the Folin-Ciocalteu method during refrigerated storage

Variables	Storage period	Phenolics	ABTS	DPPH
SB[1)]				
Storage period	1			
Phenolics	0.767***	1		
ABTS	−0.399	−0.461*	1	
DPPH	−0.721***	−0.494*	0.066	1
SL				
Storage period	1			
Phenolics	−0.720***	1		
ABTS	−0.739***	0.901***	1	
DPPH	−0.758***	0.749***	0.781***	1
KB				
Storage period	1			
Phenolics	−0.093[2)]	1		
ABTS	0.161	0.020	1	
DPPH	−0.934***	0.142	0.082	1
KL				
Storage period	1			
Phenolics	−0.806***	1		
ABTS	0.694***	−0.395	1	
DPPH	−0.955***	0.696**	−0.786***	1

[1)]SB, shinsenocho branch; SL, shinsenocho leaf; KB, kale branch; KL, kale leaf.
[2)]Pearson's coefficient by Pearson's correlation.
The correlations are significantly different at $^{*}P<0.05$, $^{**}P<0.01$, and $^{***}P<0.001$.

In conclusion, leaf parts, including kale and shinseoncho, had a more rapid decrease in total polyphenolic contents than branch parts under refrigeration. Kale was less affected than shinsheoncho in the ABTS radical scavenging capacity, whereas the DPPH radical scavenging capacity of kale showed a more rapid decrease than shinseoncho. Pearson's coefficients for the correlation between antioxidant capacities measured by the ABTS and DPPH assays, and the total polyphenolic content using the Folin-Ciocalteu method showed that the ABTS

assay (r=0.934, $P<0.001$) was more strongly positively correlated with total phenolic content than the DPPH assay (r=0.630, $P<0.001$) during refrigerated storage period. In other words, based on the findings of the present study, green vegetable juices, including kale and shinseoncho may have kept the antioxidant capacities for up to 14 days under refrigeration. The ABTS assay is superior to the DPPH assay when applied to green vegetable juices, especially in SL.

AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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