

# Influence of Cooking Methods on Bioactive Compound Content and Antioxidant Activity of Brussels Sprouts

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**ABSTRACT:** The effects of different cooking methods on total bioactive compound content were determined, and *in vitro* antioxidant activity in 80% ethanolic extracts of Brussels sprouts was evaluated by spectrophotometric methods. Compared to uncooked, steamed, and microwaved Brussels sprouts extracted with 80% ethanol contained higher amounts of total polyphenols. Uncooked Brussels sprouts contained the highest amounts of total flavonoids. Microwaved Brussels sprouts contained the highest amounts of total carotenoids (0.35 mg/g) and chlorophylls (3.01 mg/g), followed by steamed and uncooked samples. Uncooked fresh Brussels sprouts showed the highest antioxidant activity followed by microwaved and steamed sprouts. Antioxidant activity was measured with the 2,2-diphenyl-1-picrylhydrazyl, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), and hydroxyl radical scavenging assays as well as the reducing power activity assay, and antioxidant activity was found to increase in a concentration-dependent manner. Based on these results, cooking or heat treatment may decrease antioxidant activities, although their effect on bioactive compound content remains controversial.

**Keywords:** Brussels sprouts, cooking, bioactive compound, antioxidant activity

## INTRODUCTION

Brussels sprout (*Brassica oleracea* var. *gemmifera*) is a cruciferous vegetable and an important rich source of sulfur-containing compounds known as glucosinolates (1). Glucosinolates are hydrolyzed to isothiocyanates (ITCs) by the action of myrosinase in the presence of water (2). Cruciferous vegetables including Brussels sprouts contain a variety of glucosinolates, each of which forms different ITCs such as sulforaphane, phenylethyl isothiocyanates, and allyl isothiocyanates, as well as indole compounds (3). ITCs, which are potentially bioactive components present in cruciferous vegetables, are being investigated for their anticarcinogenic properties, including their ability to induce phase I and II detoxification enzymes and inhibit genes that promote tumor formation (4). Cruciferous vegetables are also good sources of phenolic compounds, flavonoids, and carotenoids (5,6).

Particularly, Brussels sprouts are among the top 20 most nutritious foods according to their Aggregate Nutrient Density Index score, which measures vitamin, mineral, and phytonutrient contents in relation to caloric content (7). Brussels sprouts also contain a high amount of chlorophyll, which can block the carcinogenic effects of heterocyclic amines generated upon grilling meats at

high temperature (8). Recent studies have reported that cruciferous vegetables including Brussels sprouts may protect humans against oxidative stress, several cancers, cardiovascular disease, diabetes, and hypertension as well as lower cholesterol levels (9-11).

Most cruciferous vegetables are commonly consumed after cooking such as blanching, boiling, steaming, or microwaving. Cooking techniques may improve the palatability of vegetables by softening the tissues, inactivating anti-nutritional compounds, microorganisms, and toxic materials, and forming color and flavor compounds (12). However, when comparing their biological actions and antioxidant activities *in vitro* and in humans, there is no consensus regarding the best way to consume these vegetables (13). Furthermore, studies of vegetable preparation have shown inconsistent results. Some studies reported increases in bioactive compound levels and antioxidant activity after cooking, whereas other studies reported decreases. Pellegrini et al. (14) reported that boiling and steaming significantly reduced the total antioxidant capacity of frozen broccoli, while microwaving without additional water did not. Turkmen et al. (15) reported varying antioxidant activities in green vegetables (broccoli, pepper, squash, green beans, peas, and spinach) and found that after boiling, microwaving, or steaming, the

Received 6 September 2017; Accepted 2 November 2017; Published online 31 December 2017

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total antioxidant activity increased or remained unchanged depending on the type of vegetable but not on the type of cooking.

Brussels sprouts are typically steamed, microwaved, or eaten fresh. This study examined the effects of different cooking methods such as steaming and microwaving of uncooked fresh Brussels sprouts on total bioactive compounds and evaluated the *in vitro* antioxidant activity in 80% ethanolic extracts of Brussels sprouts by spectrophotometric methods.

## MATERIALS AND METHODS

### Materials

Folin-Ciocalteu's phenol reagent, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), gallic acid, catechin, nicotinamide adenine dinucleotide, Tris-HCl, and sodium phosphate monobasic were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Hydrogen peroxide was purchased from Junsei Chemical Co., Ltd. (Tokyo, Japan) and 2-thiobarbituric acid was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). All chemicals used were of analytical grade.

### Sample preparation

Brussels sprouts are widely used in steamed or microwaved dishes, and we used cooking methods that are consistent with the traditional preparation of Brussels sprouts for comparison with uncooked samples. Brussels sprouts plants grown in Jeju, Korea in October 2015 were purchased. They were washed with water and dried with paper towels. The effect of cooking methods on bioactive compound contents and antioxidant activities in Brussels sprouts after controlled cooking by steaming and microwaving relative to uncooked Brussels sprouts were studied. For steaming, 300 g of Brussels sprouts was placed in a steamer pot (Kitchen Art Ltd., Gimpo, Korea) containing hot water for approximately 2 min, removed, and then cooled to room temperature before storage at  $-80^{\circ}\text{C}$ . For microwaving, 300 g of Brussels sprouts were placed in a plastic bag without additional water, cooked in a domestic microwave oven (Samsung Electronics, Suwon, Korea) for 2 min, and then cooled to room temperature before storage at  $-80^{\circ}\text{C}$ . For the fresh (uncooked) preparation, Brussels sprouts samples were stored at  $-80^{\circ}\text{C}$  after draining off the water. All samples were frozen at  $-80^{\circ}\text{C}$  and then freeze-dried. The freeze-dried samples were finely ground with a food grinder. Lyophilized powdered samples were stored at  $-80^{\circ}\text{C}$  until extraction. The powdered samples were extracted with 80% ethanol at  $60^{\circ}\text{C}$  for 2 h. Briefly, the powdered samples were mixed with 80% ethanol at a ra-

tio of 1:25 (g/mL). Next, the samples were extracted by filtering the mixture through a Whatman #2 filter paper (Whatman International Ltd., Maidstone, UK) with a vacuum filter; this process was repeated three times. The extracted filtrate was then evaporated using a rotary evaporator (EYELA, Tokyo, Japan) under reduced pressure at  $40^{\circ}\text{C}$ . The evaporated filtrate was redissolved in distilled water to a volume of 100 mL; the solution was then freeze-dried (Ilshin Biobase, Suwon, Korea). The samples were again freeze-dried to obtain powdered samples and stored at  $-20^{\circ}\text{C}$  until analysis.

### Determination of total polyphenol and flavonoid contents

The total polyphenol content was measured with the Folin-Ciocalteu's phenol reagent as described by Zhou et al. (16). Gallic acid was used as the calibration standard, and the total polyphenol contents were expressed as gallic acid equivalents (GAE) per gram of dry weight (mg GAE/g).

The total flavonoid contents of ethanolic extracts of Brussels sprouts were measured as described by Woisky and Salatino (17). Catechin was used as a calibration standard, and the total flavonoid contents were expressed as catechin equivalents (CE) per gram of dry weight (mg CE/g).

### Determination of total chlorophyll and carotenoid contents

The chlorophyll and carotenoid contents of Brussels sprouts were measured as described by Lichtenthaler and Buschmann (18). Freeze-dried powdered samples (20 mg) were mixed with 5 mL of dimethyl sulfoxide and incubated at  $65^{\circ}\text{C}$  for 6 h. After incubation, the mixture was centrifuged at 15,000 g for 5 min. The supernatant was collected, and the absorbance was read at 663 and 647 nm for chlorophyll *a* and *b* contents, respectively, and at 470 nm for carotenoid content. The chlorophyll and carotenoid concentrations were calculated using the following equations:

$$\text{Chlorophyll } a \text{ (mg/g extract)} = 12.25A_{663} - 2.79A_{647}$$

$$\text{Chlorophyll } b \text{ (mg/g extract)} = 21.50A_{647} - 5.10A_{663}$$

$$\text{Total chlorophyll (mg/g extract)} = 20.29A_{647} + 8.02A_{663}$$

$$\text{Carotenoid (mg/g extract)} = (1,000A_{470} - 1.82 \text{ Chl } a - 95.15 \text{ Chl } b) / 225$$

### Determination of antioxidant activity

The antioxidant activities of 80% ethanol extracts of Brussels sprouts were measured with *in vitro* spectrophotometric methods. The DPPH and ABTS radical scavenging activity of 80% ethanolic extracts of Brussels sprouts were determined as described by Thi and Hwang (19). Hydroxyl scavenging activity was measured as described

by Chung et al. (20). The  $\text{Fe}^{3+}$  reducing power of the extracts was determined as described by Oyaizu (21).

### Statistical analysis

All results are presented as the mean  $\pm$  standard deviation (SD). Statistical analysis was performed using SPSS software version 17.0 (SPSS Inc., Chicago, IL, USA). Data were compared using one-way analysis of variance ( $P < 0.05$ ) was considered significant.

## RESULTS AND DISCUSSION

### Total polyphenol and flavonoid contents

Table 1 presents the total polyphenol and flavonoid contents in Brussels sprouts exposed to different cooking methods. Fresh and microwaved samples extracted with 80% ethanol showed the highest total polyphenol contents of 115.62 and 115.87 mg GAE/g dry weight, respectively. In contrast, the lowest total polyphenol content of 109.00 mg GAE/g dry weight was obtained in the steamed samples. For total flavonoid contents, the data showed that the 80% ethanol extracts of uncooked Brussels sprouts contained higher flavonoid contents than the steamed and microwaved samples. The total flavonoid contents of Brussels sprouts after different cooking methods were 32.76~42.33 mg CE/g dry weight, with the fresh sample having higher flavonoid contents than the microwaved and steamed samples.

The different cooking methods affected both the polyphenol and flavonoid contents in Brussels sprouts extracts. Cooking can soften vegetable tissues, facilitating the extraction of phenolic compounds from the cellular matrix (22), resulting in an increase in total polyphenol content. Murador et al. (13) found that total phenolic content in raw kale was significantly decreased in boiled kale compared to the raw samples, but significantly increased after steaming and stir-frying. This is likely because phenolic compounds are lost via leaching by cooking in water (13,23). Xu et al. (24) reported significant losses in stir-fried and boiled samples, but found no differences between microwaving, steaming, and fresh-cut

methods, which are likely associated with the inactivation of polyphenol oxidase by heating to inhibit polyphenol degradation. Barakat and Rohn (25) found no significant difference between uncooked or fried/microwaved, and steamed broccoli-based bars, while the total phenolic contents in microwaved and baked samples were reduced by 11.7 and 7.9%, respectively.

### Total chlorophyll and carotenoid contents

Table 2 shows the chlorophyll *a*, *b*, and total chlorophyll and carotenoid contents in Brussels sprouts extracts. We found differences in total chlorophyll and carotenoid contents between the fresh, steamed, and microwaved samples. The microwaved sample had the highest chlorophyll *a*, *b*, and total chlorophyll content (1.53, 1.21, and 3.01 mg/g dry weight, respectively), whereas the uncooked sample had the lowest content of chlorophyll *a*, *b*, and total chlorophyll content (1.21, 0.98, and 2.41 mg/g dry weight, respectively). The total chlorophyll contents in uncooked and steamed Brussels sprouts extracted with 80% ethanol were 2.41 and 2.82 mg/g dry weight, respectively.

The highest and lowest total carotenoid content was found in the microwaved (0.35 mg/g dry weight) and steamed samples, respectively. Total carotenoid contents in uncooked and steamed Brussels sprouts were 0.34 and 0.29 mg/g dry weight, respectively. Total carotenoid contents after different cooking methods were observed in the following decreasing order: microwaved > uncooked > steamed.

The effects of different cooking techniques on carotenoid content are controversial in the literature. Pellegrini et al. (14) found decreases in total carotenoid content in boiled and microwaved broccoli and in steamed and microwaved Brussels sprouts. The difference between food matrices may be related to the varying carotenoid levels present in certain matrices. Another possible factor influencing these results is the deposition of carotenoids in different types of plastids, such as chloroplasts and chromoplasts. Carotenoids can associate with different proteins and can appear in lipid droplets or crystalline structures, depending on the variety of vegetables, indi-

**Table 1.** Total polyphenol and flavonoid contents of Brussels sprouts prepared by different cooking methods

	Total polyphenols (mg GAE/g)	Total flavonoids (mg CE/g)
Uncooked	115.62 $\pm$ 2.06 <sup>b</sup>	42.33 $\pm$ 3.64 <sup>b</sup>
Steamed	109.00 $\pm$ 0.82 <sup>a</sup>	32.76 $\pm$ 1.95 <sup>a</sup>
Microwaved	115.87 $\pm$ 1.41 <sup>b</sup>	39.09 $\pm$ 1.50 <sup>b</sup>

Data are means  $\pm$  SD of four separate experiments. Values with different letters (a,b) within the same column are significantly different at  $P < 0.05$ . GAE, gallic acid equivalent; CE, catechin equivalent.

**Table 2.** Chlorophyll and carotenoid contents of Brussels sprouts prepared by different cooking methods (unit: mg/g extract)

	Chlorophyll <i>a</i>	Chlorophyll <i>b</i>	Total chlorophylls	Total carotenoids
Uncooked	1.21 $\pm$ 0.02 <sup>a</sup>	0.98 $\pm$ 0.05 <sup>a</sup>	2.41 $\pm$ 0.06 <sup>a</sup>	0.34 $\pm$ 0.01 <sup>ab</sup>
Steamed	1.42 $\pm$ 0.03 <sup>b</sup>	1.15 $\pm$ 0.10 <sup>b</sup>	2.82 $\pm$ 0.08 <sup>b</sup>	0.29 $\pm$ 0.01 <sup>a</sup>
Microwaved	1.53 $\pm$ 0.04 <sup>c</sup>	1.21 $\pm$ 0.04 <sup>b</sup>	3.01 $\pm$ 0.03 <sup>c</sup>	0.35 $\pm$ 0.03 <sup>b</sup>

Data are means  $\pm$  SD of four separate experiments. Values with different letters (a-c) within the same column are significantly different at  $P < 0.05$ .

cating that the cellular matrix of the vegetable influences the release or retention of carotenoids (13,14).

### Antioxidant activity

We determined the radical scavenging potential of Brussels sprouts using widely used *in vitro* assays such as DPPH, ABTS, and hydroxyl radical scavenging activities, and reducing power assay. Brussels sprouts extracts significantly ( $P<0.05$ ) increasing radical scavenging activity as the sample concentration increased (50~1,000  $\mu\text{g}/\text{mL}$ ).

Table 3 shows that the DPPH radical scavenging activity of Brussels sprouts increased in a concentration-dependent manner (50~1,000  $\mu\text{g}/\text{mL}$ ). The average inhibition of DPPH radical formation in 500  $\mu\text{g}/\text{mL}$  fresh Brussels sprouts was 40.33% compared to 34.73% and 35.83% in steamed and microwaved Brussels sprouts, respectively. The DPPH radical scavenging activity of 1,000  $\mu\text{g}/\text{mL}$  uncooked sample was 69.10%, which was higher than those of steamed and microwaved samples (63.63% and 64.50%, respectively). The highest inhibition of DPPH radical formation was observed in uncooked Brussels sprouts extracted with 80% ethanol.

Table 4 shows the ABTS radical scavenging activity of Brussels sprouts after different cooking methods. ABTS radical scavenging activity of Brussels sprouts significant-

ly ( $P<0.05$ ) increased in a concentration-dependent manner (50~1,000  $\mu\text{g}/\text{mL}$ ). The 80% ethanol extracts of uncooked, steamed, and microwaved samples of Brussels sprouts showed inhibitory activities of 50.20%, 37.87%, and 41.20% at 500  $\mu\text{g}/\text{mL}$ , respectively. At 1,000  $\mu\text{g}/\text{mL}$ , uncooked, steamed, and microwaved Brussels sprouts samples had higher ABTS radical scavenging activities of 72.97%, 64.67%, and 67.00%, respectively. The highest ABTS scavenging activity was observed for uncooked Brussels sprouts extracted with 80% ethanol compared to heat treatments such as steaming and microwaving.

Table 5 shows the hydroxyl radical scavenging activity of extracts from Brussels sprouts prepared by different cooking methods. In samples extracted with 80% ethanol, uncooked Brussels sprouts showed higher hydroxyl radical scavenging activity than microwaved and steamed Brussels sprouts. There were no significant differences between microwaved and steamed Brussels sprouts in the hydroxyl radical scavenging activity. The hydroxyl radical scavenging activity of uncooked samples of 1,000  $\mu\text{g}/\text{mL}$  was 40.07%, which was higher than those of steamed and microwaved samples with values of 38.00% and 40.37%, respectively.

Table 6 shows the reducing power activity of Brussels sprouts after different cooking methods. High absorbance indicates high reducing power. The absorbance values of uncooked, steamed, and microwaved Brussels sprouts were 0.3043, 0.2487, and 0.2467 at 500  $\mu\text{g}/\text{mL}$ , respectively. The highest absorbance at 700 nm was observed in uncooked Brussels sprouts extracted with 80% ethanol at 1,000  $\mu\text{g}/\text{mL}$  ( $\text{OD}_{700}=0.3749$ ) ( $P<0.05$ ). The absorbance values of steamed and microwaved Brussels sprouts were 0.3423 and 0.3228 at 1,000  $\mu\text{g}/\text{mL}$ , respectively.

Based on our results, we propose that cooking may have negative effects because heat treatment can degrade bioactive compounds; this finding agrees with those of previous studies (26,27). Ahmed and Ali (26) reported that methanolic extracts of fresh cauliflower had significantly high DPPH radical scavenging activity (68.91%), followed by the blanched (61.83%), boiled (59.15%), and

**Table 3.** 2,2-Diphenyl-1-picrylhydrazyl radical radical scavenging activity of Brussels sprouts prepared by different cooking methods (unit: %)

Concentration ( $\mu\text{g}/\text{mL}$ )	Uncooked	Steamed	Microwaved
50	2.20 $\pm$ 0.50 <sup>aA</sup>	1.60 $\pm$ 0.78 <sup>aA</sup>	3.77 $\pm$ 0.40 <sup>bA</sup>
100	4.80 $\pm$ 0.46 <sup>nsB</sup>	5.17 $\pm$ 0.25 <sup>B</sup>	4.60 $\pm$ 0.60 <sup>B</sup>
250	22.20 $\pm$ 0.10 <sup>cC</sup>	17.37 $\pm$ 0.75 <sup>aC</sup>	18.57 $\pm$ 0.12 <sup>bC</sup>
500	40.33 $\pm$ 0.25 <sup>bd</sup>	34.73 $\pm$ 1.01 <sup>ad</sup>	35.83 $\pm$ 0.49 <sup>ad</sup>
1,000	69.10 $\pm$ 0.78 <sup>be</sup>	63.63 $\pm$ 0.07 <sup>ae</sup>	64.50 $\pm$ 0.36 <sup>ae</sup>

Data are means $\pm$ SD of four separate experiments. Values with different letters within the same row (a-c) and the same column (A-E) are significantly different at  $P<0.05$ .  
<sup>ns</sup>Not significant.

**Table 4.** 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging activity of Brussels sprouts prepared by different cooking methods (unit: %)

Concentration ( $\mu\text{g}/\text{mL}$ )	Uncooked	Steamed	Microwaved
50	5.10 $\pm$ 0.26 <sup>bA</sup>	2.23 $\pm$ 0.35 <sup>aA</sup>	2.50 $\pm$ 0.10 <sup>aA</sup>
100	8.17 $\pm$ 0.21 <sup>bb</sup>	5.50 $\pm$ 0.60 <sup>abB</sup>	5.13 $\pm$ 1.21 <sup>abB</sup>
250	27.73 $\pm$ 0.81 <sup>bc</sup>	21.03 $\pm$ 0.25 <sup>aC</sup>	21.33 $\pm$ 0.29 <sup>aC</sup>
500	50.20 $\pm$ 0.60 <sup>cd</sup>	37.87 $\pm$ 1.26 <sup>ad</sup>	41.20 $\pm$ 0.70 <sup>bd</sup>
1,000	72.97 $\pm$ 0.95 <sup>be</sup>	64.67 $\pm$ 1.27 <sup>ae</sup>	67.00 $\pm$ 1.67 <sup>ae</sup>

Data are means $\pm$ SD of four separate experiments. Values with different letters within the same row (a-c) and the same column (A-E) are significantly different at  $P<0.05$ .

**Table 5.** Hydroxyl radical scavenging activity of Brussels sprouts prepared by different cooking methods (unit: %)

Concentration ( $\mu\text{g}/\text{mL}$ )	Uncooked	Steamed	Microwaved
50	16.27 $\pm$ 0.91 <sup>bA</sup>	13.67 $\pm$ 0.84 <sup>aA</sup>	13.20 $\pm$ 1.04 <sup>aA</sup>
100	24.30 $\pm$ 1.48 <sup>nsB</sup>	21.90 $\pm$ 0.26 <sup>B</sup>	21.43 $\pm$ 2.06 <sup>B</sup>
250	35.90 $\pm$ 1.47 <sup>bc</sup>	35.30 $\pm$ 0.62 <sup>abc</sup>	33.13 $\pm$ 1.37 <sup>aC</sup>
500	39.23 $\pm$ 0.64 <sup>bd</sup>	36.07 $\pm$ 0.81 <sup>aC</sup>	34.97 $\pm$ 2.20 <sup>aC</sup>
1,000	40.07 $\pm$ 1.19 <sup>nsB</sup>	38.00 $\pm$ 1.71 <sup>D</sup>	40.37 $\pm$ 1.42 <sup>D</sup>

Data are means $\pm$ SD of four separate experiments. Values with different letters within the same row (a,b) and the same column (A-D) are significantly different at  $P<0.05$ .  
<sup>ns</sup>Not significant.

**Table 6.** Reducing power activity of Brussels sprouts prepared by different cooking methods

Concentration ( $\mu\text{g/mL}$ )	Uncooked	Steamed	Microwaved
50	0.1121 $\pm$ 0.0015 <sup>nsA</sup>	0.1134 $\pm$ 0.0078 <sup>A</sup>	0.1161 $\pm$ 0.0008 <sup>A</sup>
100	0.1279 $\pm$ 0.0008 <sup>nsB</sup>	0.1207 $\pm$ 0.0103 <sup>A</sup>	0.1272 $\pm$ 0.0023 <sup>B</sup>
250	0.2126 $\pm$ 0.0034 <sup>bc</sup>	0.1830 $\pm$ 0.0008 <sup>ab</sup>	0.1844 $\pm$ 0.0010 <sup>ac</sup>
500	0.3043 $\pm$ 0.0012 <sup>bd</sup>	0.2487 $\pm$ 0.0039 <sup>ac</sup>	0.2467 $\pm$ 0.0008 <sup>ad</sup>
1,000	0.3749 $\pm$ 0.0034 <sup>ce</sup>	0.3423 $\pm$ 0.0098 <sup>bd</sup>	0.3228 $\pm$ 0.0009 <sup>ae</sup>

Data are means $\pm$ SD of four separate experiments.

Values with different letters within the same row (a-c) and the same column (A-E) are significantly different at  $P < 0.05$ .

<sup>ns</sup>Not significant.

microwaved (58.24%) extracts. During heating of cruciferous vegetables, qualitative changes, antioxidant breakdown, and their leaching into surrounding water may decrease their antioxidant activity (21). Microwaved samples showed higher antioxidant activities than steamed or boiled samples because microwaving does not stimulate the release of antioxidants from cooked tissues (27, 28).

However, some studies reported that cooking processes can have positive effects, as cooking softens vegetable tissues, facilitating the extraction of bioactive compounds. The effects of cooking on antioxidant activity of cruciferous vegetables differ between vegetables and even between studies. Murador et al. (13) reported that raw kale exhibited the highest carotenoid content among all samples analyzed, while steamed red cabbage showed the highest anthocyanin concentration. Steaming resulted in maximum levels of total phenolic content. In the antioxidant activity assays, steamed kale displayed the highest antioxidant activity; however, these results were heterogeneous for red cabbage. They confirmed that the effects of heat treatment on the antioxidant effects of cruciferous vegetables differ for different vegetables.

In conclusion, Brussels sprouts contains bioactive compounds such as polyphenols, flavonoids and chlorophyll, and these may contribute to the antioxidant activity of Brussels sprouts. Fresh Brussels sprouts had higher antioxidant activities than steamed or microwaved broccoli, and this shows that cooking or heat treatment may decrease bioactive compounds and antioxidant activities.

## ACKNOWLEDGEMENTS

This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF-2016R1A2B4014977).

## AUTHOR DISCLOSURE STATEMENT

The author declares no conflict of interest.

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