



# Comparative analysis of active components and antioxidant activities of Brussels sprouts (*Brassica oleracea* var. *gemmifera*) and cabbage (*Brassica oleracea* var. *capitata*)

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**Abstract** The applicability of Brussels sprouts, which are widely cultivated in Jeju, as a functional biomaterial in the cosmetics and food industries is investigated. The active ingredients (total phenols and flavonoids,  $\beta$ -carotene, vitamin C, free sugar, minerals, glucosinolates, and isothiocyanates) and antioxidant activities of 70% ethanol extracts of Brussels sprouts and cabbage were analyzed. The total phenol, flavonoid, vitamin C, and  $\beta$ -carotene contents of Brussels sprouts were approximately 36%, 2.5 times, 2.3 times, and 65% higher than those of cabbage, respectively. The total free sugar content of Brussels sprouts was 58%-72% lower than that of cabbage. The K content was the highest among the other minerals, and there was little difference between the two samples. The Na content was approximately three times higher in cabbage than in Brussels sprouts. The total glucosinolate content of Brussels sprouts was 34.5  $\mu\text{mol SE/g DW}$ , twice that of cabbage. The sinigrin content of Brussels sprouts was thrice (10.06  $\mu\text{mol/g DW}$ ) that of cabbage. The isothiocyanate (sulforaphane, I3C) content in Brussels sprouts was double that of cabbage. The antioxidant activity of Brussels sprout extract was 2.5 to 2.8 times higher in 1,1-Diphenyl-2-picrylhydrazyl

radical scavenging capacity and 3.3 to 3.6 times higher in 2,2'-Azino-bis(3-ethylthiazoline-6-sulfonic acid) radical scavenging capacity than those of cabbage extract. These are important basic data for the study of glucosinolates and isothiocyanates, which have anticancer activity, as well as antioxidant-related substances of Brussels sprout, which has high potential for use as a biomaterial in functional foods and cosmetics.

**Keywords** Antioxidant-activity · Brussel sprouts · Glucosinolates · Isothiocyanates · Total phenols

## Introduction

Brussel sprouts (*Brassica oleracea* var. *gemmifer*) is similar in appearance to ordinary cabbage (*Brassica oleracea* var. *capitata*), but it is called “Brassian cabbage” or “mini cabbage” because approximately 30 g of the latter is attached to a long stem, similar to a bell [1,2]. Brussels sprouts are widely cultivated in Europe and the United States, and it a cruciferous vegetable that is widely consumed in winter because of its superior taste, quality, and excellent storage properties [3]. Brussels sprouts were first introduced in Korea in the 1990s, and they have been cultivated in small amounts in Jeju; however, with the recent increase in their consumption, the cultivated area has increased significantly.

Cruciferous vegetables, including Brussels sprouts, broccoli, and cabbage, are rich in minerals such as potassium and iron as well as functional ingredients such as vitamin C, vitamin U, carotenoids, and polyphenols [4,5]. In particular, cruciferous vegetables contain glucosinolates and isothiocyanates, which are highly volatile and spicy with a unique aroma, thereby preventing stomach cancer, liver cancer, and lung cancer [6,7]. Cruciferous vegetables contain approximately 100 types of glucosinolates,

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mainly glucobrassicinapin, glucoraphanin, gluconapin, and sinigrin [8]. The total glucosinolate content of cruciferous vegetables is affected by the type and variety of vegetables, growing conditions, harvest time, and climate [9,10].

In general, Brussels sprouts are known to be rich in minerals such as iron and more than twice as high in vitamins A and C than cabbage, so they are considered to have high potential for use as a raw material for various functional foods and cosmetics. However, research on the physicochemical components or efficacy of Brussels sprouts for the development of functional materials is insufficient. Therefore, this study attempted to evaluate the superiority of Brussels sprouts as a functional biomaterial by comparing and evaluating their active ingredients and antioxidant activity with those of cabbage. For this purpose, the amounts of antioxidant-related substances (total polyphenol, total flavonoids, vitamin C,  $\beta$ -carotene), free sugars, minerals, and the major glucosinolates and isothiocyanates in Brussels sprouts and cabbage extracts were analyzed. In addition, the antioxidant activities of these extracts were compared.

## Materials and Methods

### Materials

The Brussel sprouts (Brilliant variety) and cabbage (Green 345 and Harutama varieties) used in this study were harvested in Aewol-eup, Jeju-do, in February 2021 and stored at low temperature ( $0\pm 2^\circ\text{C}$ ) for one week. Samples without external wounds were selected. The samples were cut, dried in a freeze dryer (HC-3055, Bio-Medical Science Co., Ltd., Seoul, Korea), ground into powder form, and stored at  $-20^\circ\text{C}$ . Sinigrin, a standard for glucosinolate analysis, was purchased from Abcam (Cambridge, MA, USA). Gluconapin, glucoraphenin, gluconastrutiin, and glucobrassicinapin were procured from Phytolab GmbH & Co., KG (Dutendorfer, Germany). Glucotropaeolin was purchased from Extrasynthese (Impasse Jacquard, Genay, France). The standard for sulforaphane analysis was purchased from Cayman Chemical (Ann Arbor, MI, USA), and the indole-3-carbinol standard was purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Reagents for the analysis of physiologically active substances and determination of antioxidant activity, and the solvents for HPLC analysis were purchased from Fisher Scientific (Fairlawn, NJ, USA) and Sigma-Aldrich Co..

### Active ingredient analysis

**Total polyphenol and flavonoid analysis:** As per the method proposed by Jeon et al. [11], 10 mL of 70% ethanol was added to 1 g of dry sample powder, followed by ultrasonication for 30 min in an ultrasonic device (Power sonic 420, Hwashin Technology, Seoul, Korea) for extraction. Then, centrifugation (3,500 rpm, 5 min) was performed at room temperature (Felta 40, HANIL Science Co., LTD, Gimpo, Korea), and the supernatant was

filtered through Advantec Filter Paper No. 2.

The filtrate was concentrated on a vacuum centrifugal concentrator (JP/CVE-3110, Eyla, Tokyo, Japan) and dissolved in 10 mL of DMSO. The total polyphenol content was measured according to the experimental method proposed by Jeon et al. [11], with reference to the method reported by Folin and Denis [12]. Briefly, 100  $\mu\text{L}$  of the sample extract was mixed with 400  $\mu\text{L}$  of distilled water, and then, 200  $\mu\text{L}$  of 1N Folin-Ciocalteu reagent was added and mixed, followed by reaction in the dark for 3 min. After 3 min of incubation, 300  $\mu\text{L}$  of 10%  $\text{Na}_2\text{CO}_3$  was added to the reaction mixture, which was then incubated at room temperature for 30 min in the dark. Following incubation, absorbance was measured at 750 nm using a microplate reader. TPC is expressed as milligrams of quercetin equivalent (QE) per gram of dry weight. The total flavonoid content of the extracts was determined using a method previously described by Moreno et al. [13]. Briefly, 40  $\mu\text{L}$  of the extract was mixed with 80  $\mu\text{L}$  distilled water and 6  $\mu\text{L}$  5%  $\text{NaNO}_2$ . After 5 min of incubation, 12  $\mu\text{L}$  of 10%  $\text{AlCl}_3$  was added to the reaction mixture and incubated at room temperature for 5 min. Following incubation, 40  $\mu\text{L}$  of 1 N NaOH was added to the reaction mixture, and the absorbance was measured at 510 nm using a microplate reader. TFC is expressed as milligrams of QE per gram of dry weight.

**$\beta$ -Carotene analysis:** The  $\beta$ -carotene content of the samples was analyzed by HPLC according to the method of Jeon et al. [11]. The solvent (EtOH + 0.1% ascorbic acid (w/v), 3 mL) was added to 0.1 g a freeze-drying sample, and after 5 min of heat treatment at  $85^\circ\text{C}$ , 80% KOH 120  $\mu\text{L}$  was added, followed by reaction at  $85^\circ\text{C}$  for 5 min. After saponification, 1.5 mL of cold distilled water was added to cool the mixture; then, 3 mL of hexane was added, followed by stirring for a sufficient time, and centrifugation (3500 rpm, 5 min) at  $4^\circ\text{C}$  to obtain the supernatant. This extraction process was repeated three times, and the extracts were dried using a speed-vacuum. Then, 3 mL of a solvent (dichloromethane/MeOH = 50:50, 0.1% ascorbic acid) was added to dissolve the residue, and the solution was used for  $\beta$ -carotene analysis. For the quantitative analysis of  $\beta$ -carotene, an HPLC system (Shimadzu Corp, Kyoto, JAPAN) was used, and separation was performed on a Shim-pack GIS C18 column (4.6 $\times$ 250 mm, 5  $\mu\text{m}$ , Shimadzu, Kyoto, Japan).  $\beta$ -carotene detection was measured at 450 nm using a PAD detector. The mobile phases were solvent A (acetonitrile/methanol/dichloromethane: 70:20:5, v/v/v) and solvent B (acetonitrile/methanol/dichloromethane: 70:10:30, v/v/v), which were flowed by gradient elution at a flow rate of 1 mL/min. For the first 3.5 min, 100% solvent A was allowed to flow, and then solvent B was allowed to flow to 100% for 22 min. Subsequently, 100% of solvent B was maintained for 28.5 min, then 100% of solvent A was flowed for 30 min, and 100% of solvent A was maintained for 40 min.

**Vitamin C and free sugar analysis:** The freeze-dried powder (1 g) was extracted with 20 mL of 5% metaphosphoric acid (w/v) and 10 mM EDTA (w/v) for 10 min at room temperature. The

extracts were centrifuged at 3,500 rpm for 10 min at room temperature. The collected supernatants were filtered using a 0.2 µm nylon syringe filter and then analyzed for vitamin C. HPLC analysis was carried out on a Shim-pack GIS C18 column at a flow rate of 0.6 mL/min, and the mobile phase was 0.1% trifluoroacetic acid. The injection volume of the sample was 20 µL, and vitamin C was measured at 254 nm using a PDA detector.

The free sugar content was measured using the method described by Oh et al. [14]. Accordingly, 50% ethanol (25 mL) was added to the sample (5 g); then, the sugars were extracted at 85 °C for 25 min and centrifuged (2,000 rpm, 10 min). The supernatant was filtered through a 0.45 µm membrane filter (Woongki Science Co. Ltd., Seoul, Korea) and then analyzed by HPLC (Waters 2695, Waters Associate Inc., Milford, MA, USA). A Prevail™ Carbohydrate ES (4.6×250 mm, 5 µm, Grace, Deerfield, MA, USA) column and a refractive index (RI) detector were used for the analysis of free sugar. The flow rate of the mobile phase (acetonitrile/distilled water = 75:25) was 1.0 mL/min.

**Mineral analysis:** According to the AOAC method [15], 8 mL of 20% HNO<sub>3</sub> was added to the sample (0.5 g), and wet decomposition was performed using a microwave oven. After the wet decomposition was complete, the sample was filtered and adjusted to 50 mL using distilled water and then used as an analytical solution. Mineral analysis was performed by ICP-OES (Perkin Elmer, Optima 8300) using 1% HNO<sub>3</sub> as a control. The analysis conditions were as follows for the determination of Na (589.5 nm), Ca (317.9 nm), K (766.5 nm), Fe (238.204 nm), and Mg (285.2 nm): RF power, 1,300 W; plasma flow, 12.0 L/min; auxiliary flow, 0.20 L/min; nebulizer flow, 0.50 L/min; and pump RPM, 1.5 mL/min.

**Glucosinolate analysis:** The total glucosinolate content was measured using the method described by Mawlong et al. [16]. The extraction was repeated three times at 70 °C for 15 min by adding 80% MeOH (1 mL) to 0.1 g of freeze-dry powder sample. The extracted samples were diluted twice after centrifugation (3000 rpm, 5 min) and used for analysis. Distilled water (0.3 mL) and 2 mM sodium tetrachloropalladate were added to 100 µL of the samples, and the absorbance was measured at 425 nm after 1 h of response at room temperature. The total glucosinolate content was calculated using sinigrin as the standard, and expressed as µg sinigrin equivalent (µg SE/g DW). The single glucosinolate content of the sample was measured using the HPLC method proposed by Kim et al. [17] according to the ISO 9167-1 method [18]. The crude glucosinolates were extracted with 70% boiling methanol (1.5 mL) from lyophilized powder (100 mg) in a water bath at 70 °C for 5 min. The mixture was centrifuged at 3,500 rpm for 10 min at 4 °C. The residue was re-extracted twice using the same procedure, and the supernatants were collected. The crude glucosinolate extract was applied to a mini-column using a 1 mL pipet tip packed with DEAE-Sephadex A-25, and then, the glucosinolates were desulfated by adding aryl sulfatase solution

(75 µL, 29 units) to the column. After an overnight reaction at room temperature, the desulfoglucosinolates were eluted with 5 mL distilled water. The solution was then filtered through a 0.45 µm hydrophilic PTFE syringe filter (Ø 13 mm, Advanter, Tokyo, Japan) in a brown vial. The glucosinoalte content of the Brussels sprout extracts was analyzed by HPLC on a Shim-pack GIS C18 column. The analysis conditions were as follows: column oven temperature, 40 °C; flow rate, 1.0 mL/min; and mobile phase, acetonitrile (A) + water (B). The injection volume was 20 µL, and the glucoseinolates were measured at 229 nm using a PDA detector. Gradient elution started until a 100% mobile phase composition of B. Over 30 min, gradient elution was performed until the mobile phase composition was 30% A and 70% B. Over 5 min, gradient elution was performed until the mobile phase composition was 0% A and 100% B. Gradient elution was performed for 5 min.

**Sulforaphane and indole-3-carbinol (I3C) analysis:** The sulforaphane and indole-3-carbinol contents of the samples were determined according to the procedure described by Kim et al. [19] with some modifications. Briefly, 1 g of each sample was mixed with 3 mL of distilled water and vortexed for 10 min at room temperature. The mixture was allowed to rest for 10 min; then, 15 mL of dichloromethane was added, and the mixture was vortexed and incubated at room temperature for 30 min. The extracts were centrifuged at 3500 rpm for 10 min. The supernatant was filtered through filter paper containing anhydrous sodium sulfate. The extraction was repeated again twice, and the extracts were combined. The collected supernatant was evaporated under vacuum at 40 °C and dissolved in 2 mL acetonitrile. The extracts were filtered and analyzed by an HPLC system equipped with an ultraviolet detector (Shimadzu CBM-20A, Tokyo, Japan) comprising a CBM-20A system controller, an SIL-20A autosampler, an SPD-M20A diode array detector, an LC-20AD solvent delivery unit, a DGU-20A3R degassing unit, and a CTO-20A column oven. The extracts (20 µL) were separated on a Shim-pack GIS C18 column at 40 °C with a gradient solvent system consisting of water/acetonitrile (80:20 to 0:100 v/v) for 50 min. The flow rate was 0.8 mL/min. The detection wavelength was 205 nm for sulforaphane and 279 nm for indole-3-carbinol (I3C).

#### **Antioxidant activity assay**

1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical and 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging activity assays were performed as previously described, with slight modifications [20].

The radical scavenging activity was calculated using the following equation:

$$\text{Radical scavenging activity (\%)} = [1 - (\text{Abs}_{\text{sample}}/\text{Abs}_{\text{blank}})] \times 100.$$

The sample concentration that led to 50% inhibition concentration (IC<sub>50</sub>) was calculated from the graph of inhibition percentage against sample concentration.

### Statistical analysis

All experiments were performed in triplicate. Data are presented as mean  $\pm$  SD. One-way analysis of variance (ANOVA) was performed using SPSS version 20 (IBM, New York, USA). Differences among the sample means were evaluated using Dunnett's multiple comparison test at the 95% confidence level ( $p < 0.05$ ).

## Results and Discussion

Phenolic compounds present in plants have various derivatives. Polyphenols containing flavonoids are known to show strong antioxidant activities, in addition to physiological activities such as anti-inflammatory and anticancer activities, as well as hypertension inhibition activity [21,22]. Therefore, the amounts of antioxidant substances in Brussels sprouts and their relationship with the antioxidant activity were investigated. For this purpose, the antioxidant content (total polyphenol, total flavonoid, vitamin C, and  $\beta$ -carotene) of the ethanol extracts of Brussels sprouts and cabbage was investigated (Table 1). The total polyphenol and flavonoid contents of the Brussels sprouts were 5.34 mg and 4.68 mg, respectively, based on QE per 1 mg of dry weight. On the other hand, the total polyphenol content of cabbage was 3.78 mg QE/g DW and 4.04 mg QE/g DW in Green 345 and Harutama varieties, respectively. The corresponding flavonoid contents were 1.40 mg QE/g DW and 1.75 mg QE/g DW, respectively. The results of this study showed that the polyphenol and flavonoid contents in Jeju Brassica vegetables were within the previously reported ranges [23-25]. The TPC content (83.8 mg/100 g (fw)) of Brussels sprouts obtained in this study was somewhat lower than that of previous studies, but the TPC content (44.6-48.0 mg/100 g

FW) of cabbage was within the range of previous studies. The slight difference in TPC and TFC content between cruciferous vegetables obtained in this study and previous studies is considered to be due to the cultivation environment, because the secondary metabolites of plants are generally known to be affected by cultivars, cultivation environment, and harvest time [10,11]

The total polyphenol and flavonoid contents of Brussels sprouts (Brilliant) were higher than those of Green 345 and Harutama sprouts. The total polyphenol content of Brussels sprouts was 32%-41% higher than that of cabbage, and the total flavonoid content was approximately 2.2-2.8 times higher. On the other hand, the vitamin C content of Brilliant was 465.90 mg/100 g DW, which was about 52% and 2.3 times higher than those in Green 345 and Harutama, respectively. In general, the vitamin C content of Brussels sprouts is known to be in the range of 66-196 mg/100 g FW, and in special cases, up to 284 mg/100 g FW, which is inversely proportional to their head size [26,27]. In addition, the  $\beta$ -carotene content of Brussels sprouts was approximately 67.86  $\mu$ g/g DW, which was approximately 61-70% higher than that of cabbage. Consequently, the amount of antioxidant-related substances in Brussels sprouts was higher than that in cabbage, and these antioxidants, together with glucosinolates, are thought to contribute to antioxidant and anticancer activities.

Table 2 shows the free sugar contents of Brussels sprouts and cabbage extracts. Fructose, glucose, and sucrose were detected, but not maltose. The total free sugar contents of Brilliant, Green 345, and Harutama were 28.73, 49.47, and 45.53 g/100 g DW, respectively, and the free sugar content of Brussels sprouts was lower than that of cabbage. On the other hand, in Brussels sprouts, the sucrose content was 12.37 g/100 g DW, which was the highest at 43% of total free sugar. In cabbage, the glucose and fructose

**Table 1** Antioxidants content of Brussels sprouts and cabbage

Cultivar	Total phenols (mg QE/g DW)	Total flavonoids (mg QE/g DW)	Vitamin C (mg/100 g DW)	$\beta$ -carotene ( $\mu$ g/g DW)
Brilliant	5.34 $\pm$ 0.24***	4.68 $\pm$ 0.44***	465.90 $\pm$ 3.08***	67.86 $\pm$ 2.13***
Green 345	3.78 $\pm$ 0.17	1.17 $\pm$ 0.07	306.94 $\pm$ 5.90	42.11 $\pm$ 1.64
Harutama	4.04 $\pm$ 0.19	1.46 $\pm$ 0.14	206.43 $\pm$ 2.55***	39.87 $\pm$ 1.61

Data were expressed as mean  $\pm$  SD for three tests

A significant difference was determined by one-way ANOVA with Dunnett's test

\*\*\* $p < 0.001$  compared with control (Green 345)

**Table 2** Monosaccharide content of Brussels sprouts and cabbage

Cultivar	Fructose	Glucose	Sucrose	Maltose	Total
	g/100 g DW				
Brilliant	7.83 $\pm$ 0.06***	8.53 $\pm$ 0.06***	12.37 $\pm$ 0.06***	ND	28.73 $\pm$ 0.15***
Green 345	19.17 $\pm$ 0.15	20.20 $\pm$ 0.17	10.10 $\pm$ 0.26	ND	49.47 $\pm$ 0.38
Harutama	17.07 $\pm$ 0.15***	19.67 $\pm$ 0.06**	8.80 $\pm$ 0.10***	ND	45.53 $\pm$ 0.25***

Data were expressed as mean  $\pm$  SD for three tests

A significant difference was determined by one-way ANOVA with Dunnett's test

\*\* $p < 0.01$ , \*\*\* $p < 0.001$  compared with control (Green 345)

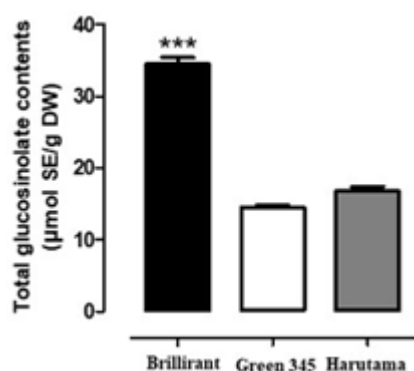
**Table 3** Content of minerals of Brussels sprouts and cabbage

Cultivar	Na	Ca	K	Fe	Mg
	mg/100 g DW				
Brilliant	30.17±0.47***	139.73±7.86	1389.47±53.51	6.07±1.78	141.17±2.95***
Green 345	92.33±2.91	182.00±25.94	1355.37±77.56	5.47±0.40	125.17±1.93
Harutama	95.40±3.11	248.17±18.30**	1307.30±102.59	3.90±0.17	139.43±2.08***

Data were expressed as mean±SD for three tests

A significant difference was determined by one-way ANOVA with Dunnett's test

\*\* $p < 0.01$ , \*\*\* $p < 0.001$  compared with control (Green 345)



**Fig. 1** Total glucosinolates content of Brussels sprouts (Brilliant) and cabbage (Green 345, Harutama) extracts. Data were expressed as mean±SD for three tests. A significant difference was determined by one-way ANOVA with Dunnett's test. \*\*\* $p < 0.001$  compared with control (Green 345)

contents were higher than the sucrose content, as reported by Rosa et al. [28]. The mineral contents of Brussels sprouts and cabbage are listed in Table 3. Among the five inorganic materials analyzed, the K content was the highest at 1,307-1,389 mg/100 g DW, while the Fe content was the lowest at 3.90-6.07 mg/100 g DW. The Na content of cabbage was 92.33-95.40 mg/100 g DW, which was more than thrice of Brussels sprouts.

The total glucosinolate content of the Brussels sprout and cabbage extracts was analyzed by spectrophotometry (Fig. 1). The total glucosinolates content of Brussels sprouts was 34.5 µmol SE/g DW, which was about 2.1 to 2.4 times higher than that of cabbage (14.44 to 16.77 µmol SE/g DW). Our results were similar to those reported by Hwang et al. [28], according to which the total glucosinolate contents of Chinese cabbage and cabbage were

13.55 and 12.52 µmol/g, respectively. The amounts of gluconapin, gluconasturtiin, glucotropaeolin, sinigrin, glucobrasicanapin, and glucoraphenin, which are the main glucosinolates in cruciferous vegetables, were analyzed by HPLC (Table 4). Among the six glucosinolates, gluconapin, gluconasturtiin, glucotropaeolin, and sinigrin were detected, whereas glucobrasicanapin and glucoraphenin were not detected. The sinigrin content of Brussels sprouts was the highest (10.06 µmol/g DW) among the glucosinolates analyzed, followed by gluconapin, glucotropaeolin, and gluconasturtiin (1.70, 0.53, and 0.51 µmol/g DW, respectively). The sinigrin content of cabbage was 2.10 µmol/g DW in Green 345 and 3.02 µmol/g DW in the Harutama variety, which was lower than that (4.54 µmol/g) in the sinigrin content reported by Hwang et al. [29]. In both Brussels sprouts and cabbage, sinigrin was the main glucosinolate, consistent with Kushad's results [10]. They reported that the main glucosinolates in cabbage were sinigrin, glucoapin, glucobrassicin, and progoitrin, and that the gluconasturtiin content was 0.5 µmol/g DW. The glucosinolates of cruciferous vegetables vary depending on the type of vegetable as well as the varieties, cultivation environment, and cooking processing conditions [30,31].

On the other hand, cruciferous vegetables contain about 120 kinds of glucosinolates with various physiological activities. These glucosinolates are hydrolyzed by myrosinase when the tissue of the plant is injured by pests or during harvesting or cooking, and are decomposed into isothiocyanate, nitrile, thiocyanate, and glucose [8]. Sulforaphane (*S*-methylsulfanylbutyl isothiocyanate) is produced together with sulfuraphane nitrile upon decomposing glucoraphenin, a precursor, by myrosinase when plant tissues are damaged [32]. Sulforaphane is known to prevent cancer through cancer cell apoptosis, cell cycle regulation, and anticancer activity,

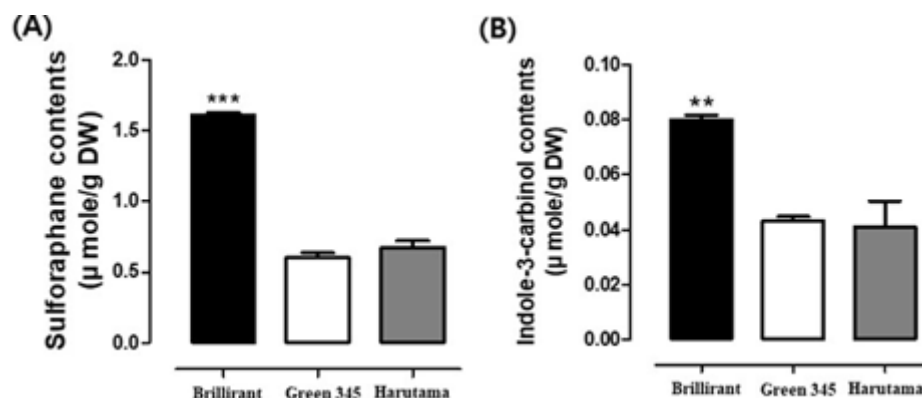
**Table 4** Glucosinolates content of Brussels sprouts and cabbage

Cultivar	Glucosinolates content (µmol /g DW)					
	Glucobrassicinapin	Gluconapin	Gluconasturtiin	Glucoraphenin	Glucotropaeolin	Sinigrin
Brilliant	ND	1.70±0.13	0.51±0.00*	ND	0.53±0.01	2.10±0.15
Green 345	ND <sup>1)</sup>	1.56±0.06	0.55±0.01	ND	0.49±0.03	2.10±0.15
Harutama	ND	1.18±0.04**	0.71±0.03***	ND	0.46±0.02	3.02±0.29

Data were expressed as mean±SD for three tests

A significant difference was determined by one-way ANOVA with Dunnett's test

\* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$  compared with control (Green 345)



**Fig. 2** (A) Sulforaphane and (B) indole-3-carbinol content of Brussels sprouts (Brilliant) and cabbage (Green 345, Harutama) extracts. Data were expressed as mean  $\pm$  SD for three tests. A significant difference was determined by one-way ANOVA with Dunnett's test. \*\* $p$  < 0.01, \*\*\* $p$  < 0.001 compared with control (Green 345)

**Table 5** The antioxidant activity of Brussels sprout and cabbage using DPPH assay (IC<sub>50</sub>) and ABTS assay (IC<sub>50</sub>)

Cultivar	DPPH IC <sub>50</sub> (µg/mL)	ABTS IC <sub>50</sub> (µg/mL)
Brilliant	1510.05 $\pm$ 27.81***	133.77 $\pm$ 0.99***
Green 345	4310.01 $\pm$ 29.17	477.38 $\pm$ 6.19
Harutama	3838.29 $\pm$ 26.68***	432.85 $\pm$ 3.05***

Data were expressed as mean  $\pm$  SD for three tests

A significant difference was determined by one-way ANOVA with Dunnett's test

\*\*\* $p$  < 0.001 compared with control (Green 345)

and it exerts strong antibacterial effects against *Helicobacter* [33–35]. In addition, derivatives such as indole-3-carbinol (I3C) and 3,3'-di-indolmethane contained in cruciferous vegetables are produced through the cutting, decomposition, and cooking processes of plant tissue; these derivatives also have excellent cancer prevention effects [36]. Figure 2 shows the results for the HPLC analysis of sulforaphane and I3C in Brussels sprouts. The sulforaphane content of Brussels sprouts was 1.61 µmol /g DW, which was 2.4 to 2.7 times higher than that of cabbage. The I3C content of Brussels sprouts was twice that of cabbage, showing 0.08 µmol/a DW.

The antioxidant activity of Brussels sprouts was measured by DPPH and ABTS radical scavenging capacity; the IC<sub>50</sub> values are shown in Table 5. The ABTS radical scavenging activity revealed a higher IC<sub>50</sub> value than the DPPH scavenging activity. This is consistent with a report that ABTS radicals can react well with various types of antioxidants because they react with both polar and non-polar substances, unlike DPPH radicals [37]. The IC<sub>50</sub> value of the DPPH radical scavenging activity of the ethanol extract was 1.51 mg/mL, and the antioxidant activity was 2.5 to 2.8 times higher than that of cabbage (3.83 to 4.31 mg/mL). Based on the ABTS radical scavenging activity, Brussels sprouts showed approximately 3.2 to 3.6 times higher antioxidant activity than did

cabbage sprouts. This result is consistent with the report that the amounts of antioxidants such as total polyphenols show a strong correlation with the radical scavenging ability [38].

Taken together, the results of this study suggest that Brussels sprouts contain not only glucosinolates and isothiocyanates, which are representative functional components of cruciferous vegetables, but also antioxidants (total polyphenols and flavonoids, vitamin C, and beta-carotene), which are considered to be useful raw materials for functional biomaterials.

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