Food Chemistry 199 (2016) 612–618

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Antioxidant capacities and polyphenolics of Chinese cabbage (*Brassica rapa* L. ssp. Pekinensis) leaves

Gi-Un Seong [a], In-Wook Hwang [b], Shin-Kyo Chung [a,*]

[a] School of Food Science and Biotechnology, Kyungpook National University, Daegu 702-701, Republic of Korea
[b] Food and Bio-industry Research Institute, Kyungpook National University, Daegu 702-701, Republic of Korea

ARTICLE INFO

Article history:
Received 19 August 2015
Received in revised form 9 December 2015
Accepted 14 December 2015
Available online 15 December 2015

Keywords:
Chinese cabbage leaves
Antioxidants
Polyphenolics
Sinapic acid
Principal component analysis

ABSTRACT

Chinese cabbage (*Brassica rapa* L. ssp. Pekinensis) is a green leafy vegetable used mainly in kimchi, salted and fermented dishes. Consumer preference for the leaf portion differs according to the type of dishes. In this study, Chinese cabbage was divided into three parts, and their antioxidant activities were investigated through *in vitro* assays. The total phenolic contents (TPC), total flavonoid contents (TFC), and vitamin C contents were also determined as indicators of antioxidant contents. The phenolic acids and flavonoids were separated and identified using high performance liquid chromatography (HPLC) and liquid chromatography/mass spectrometry (LC/MS). The outer leaf had the strongest antioxidant activity with the maximum antioxidant contents, followed by the mid- and inner leaves. Principal component analysis (PCA) revealed that outer leaf is positively related to caffeic acid, *p*-coumaric acid, ferulic acid, and myricetin contents, whereas the mid- and inner leaves are negatively related to sinapic acid contents.

1. Introduction

Chinese cabbage (*Brassica rapa* L. ssp. Pekinensis) is a cruciferous green leafy vegetable consumed primarily in Asian countries (Kim & Klieber, 1997), although its consumption has gradually increased in Western countries (Islam & Choi, 2008). Green leafy vegetables including cabbage, broccoli, kale, spinach, mustard greens, and lettuce are high in fiber, vitamins, and minerals have become popular components of balanced diets worldwide (Oboh, Raddatz, & Henle, 2008; Subhasree, Baskar, Laxmi Keerthana, Lijina Susan, & Rajasekaran, 2009). In Korea, Chinese cabbage is the principal ingredient of kimchi, which is a salted and fermented dish including whole cabbage leaves. However, the consumer preference for a particular part of Chinese cabbage varies among recipes considering the appearance, taste, and texture. In some foods, the outer greenish leaves are used for soups with soybean paste, whereas the yellowish inner leaves are used as vegetable outer rolls for grilled pork, beef, chicken, and seafood dishes. With respect to its health benefits, the dietary antioxidants in Chinese cabbage have been extensively studied for their ability to prevent reactive oxygen species (ROS), which mediate aging and oxidative damage-inducing pathological disorders such as inflammation and atherosclerosis (Huxley & Neil, 2003; Ji et al., 2011). In addition, several antioxidant phenolic compounds including flavonoids were investigated and identified in whole Chinese cabbage leaves (Harbaum et al., 2007; Kim, Padilla-Zakour, & Griffiths, 2006; Miean & Mohamed, 2001; Roy, Takenaka, Isobe, & Tsushida, 2007). Because Chinese cabbage leaves have different color, texture, taste, and availability depending on their parts, the different antioxidant capacities should be investigated to utilize as fresh-cut products and health-benefit materials.

In this study, Chinese cabbage leaves were divided into three sections, and their antioxidant activities were determined along with antioxidant contents such as phenolic acids and flavonoids. In addition, the polyphenolic profiles were differentiated by principal component analysis depending on the leaves section.

2. Materials and methods

2.1. Sample materials and extracts

Chinese cabbages (*B. rapa* L. ssp. Pekinensis cv. Hwiparam) cultivated in the field and harvested early in November 2013 in Haenam-gun, Korea, were obtained from Hwawon Agricultural Cooperative (Haenam-gun, Korea). The Chinese cabbages were divided into three different sections of inner, mid- and outer leaves, lyophilized, and ground using a roller mill (CW Brabender

* Corresponding author.
E-mail address: kchung@knu.ac.kr (S.-K. Chung).

http://dx.doi.org/10.1016/j.foodchem.2015.12.066
0308-8146/

Instruments Inc., South Hackensack, NJ, USA) (Amin & Lee, 2005; Isabelle et al., 2010; Watanabe, Musumi, & Ayugase, 2011). A cross-section of a head of Chinese cabbage is shown in Fig. 1. The outer, mid-, and inner leaves were denoted as L1, L2, and L3, respectively. After chloroform treatment for removing the color from chlorophylls, 1 g aliquots of the sample powders were extracted with 75% methanol (10 mL × 2) for 12 h at room temperature. The extracts were then filtered and dried using a rotary evaporator.

2.2. Reagents and instruments

α,α-Diphenyl-2-picrylhydrazyl (DPPH), 2,4,6-tris(2-pyridyl)-1,3,5-triazine (TPTZ), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 2,2′-azobis(2-methylpropionamidine) dihydrochloride (AAPH), gallic acid, trolox, (±)-catechin, 2,4-dinitrophenylhydrizine (2,4-DNP), ascorbic acid, sodium nitrite, aluminum chloride, caffeic acid, sinapic acid, *p*-coumaric acid, ferulic acid, and myricetin were purchased from Sigma–Aldrich Chemical Co. (St. Louis, Mo., USA). All organic solvents were of analytical grade (Merck, Darmstadt, Germany), except for HPLC (J.T. Baker, Phillipsburg, NJ, USA). An ultraviolet–visible (UV–Vis) spectrophotometer (UV 1601 PC, Shimadzu Co., Kyoto, Japan), a microplate reader (Emax Precision Microplate Reader, Molecular Devices, Sunnyvale, CA, USA), and a multi-label plate counter (Victor 3 1420, PerkinElmer Inc., Boston, MA, USA) were used for the determination of antioxidant activities and antioxidant contents. HPLC (1260 Infinity Quaternary LC System, Agilent Technologies, Palo Alto, CA, USA) and liquid chromatography/mass spectrometry (LC/MS; Agilent Technologies series 1200 system, Agilent Technologies, Palo Alto, CA, USA) were used for polyphenolics analysis and identification.

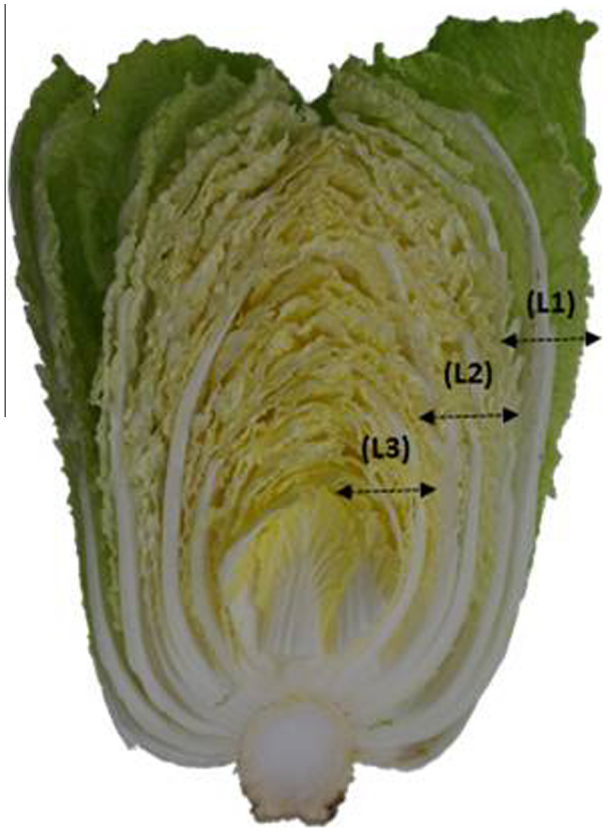

Fig. 1. Cross-section of the Chinese cabbage leaves. L1, outer leaves; L2, mid-leaves; L3, inner leaves.

2.3. Antioxidant capacities of Chinese cabbage leaves

2.3.1. DPPH radical scavenging activity

Free radical scavenging activity was measured using the DPPH method (Lim, Lim, & Tee, 2007). Nine hundred micro liter of 200 μM DPPH· solution was added to 100 μL of the crude extract of the Chinese cabbage leaves (10 mg/mL). The mixture was allowed to stand for 30 min in the dark, and then, the absorbance was measured at 490 nm. The activities were converted to μM trolox equivalents (μM TE).

2.3.2. Ferric ion reducing antioxidant power (FRAP) assay

The FRAP assay was performed using a TPTZ solution (Benzie & Strain, 1996). The working solutions were prepared by mixing 25 mL of acetate buffer (pH 3.6, 300 mM), 2.5 mL of TPTZ solution (10 mM), and 2.5 mL of $FeCl_3{\cdot}6H_2O$ solution (20 mM), and were kept at 37 °C. The sample solutions (10 mg/mL) were then mixed with the working solutions and were incubated under darkness at 37 °C for 30 min. The absorbance was measured at 650 nm, and the activities were converted to μM TE.

2.3.3. Trolox equivalent antioxidant capacity (TEAC) assay

The TEAC assay was based on the scavenging of ABTS radical anions method (Sariburun, Sahin, Demir, Türkben, & Uylaşer, 2010). The $ABTS^{+\cdot}$ solution (7 mM) was mixed with a 1:1 (v/v), potassium persulfate (2.45 mM) solution, which was kept under darkness at room temperature for 14 h. This stock solution was diluted with methanol to adjust to the absorbance value of 1.0 (±0.02) at 734 nm. The sample solutions (10 mg/mL) were mixed with the $ABTS^{+\cdot}$ working solution and were kept for 7 min. Their absorbance values were measured at 734 nm. The activities were converted to μM TE.

2.3.4. Oxygen radical absorbance capacity (ORAC) assay

The ORAC assay was measured by a modification of a previous method (Ou, Hampsch-Woodill, & Prior, 2001). Fluorescein solution (0.2 mM) was mixed with sample solutions (1 mg/mL) or phosphate buffer saline (PBS) as a blank or Trolox (0.25–2 mM) as a standard. After adding the AAPH· solution (200 mM), the fluorescence was measured every 5 min for 120 min with excitation and emission at 485 nm and 530 nm, respectively. The area under the fluorescence decay curve (AUC) was calculated by applying the following formula:

$$\text{AUC} = \left(0.5 + \sum_{i=1}^{i=120} f_i/f_0\right) \times 5 \quad \text{net AUC} = \text{AUC}_{\text{sample}} - \text{ACU}_{\text{Blank}}$$

where f_0 is the initial fluorescence at 0 min, and f_i is the fluorescence at i min. ORAC-FL values were converted to μM TE.

2.4. Antioxidant contents of the Chinese cabbage leaves

2.4.1. Total phenolic contents (TPC)

The TPC was measured using Folin–Ciocalteu reagent (Singleton, Orthofer, & Lamuela-Raventos, 1999). The contents were converted to milligram of gallic acid equivalent (GAE) per 100 gram of dry weight basis (mg GAE/100 g dwb).

2.4.2. Total flavonoid contents (TFC)

The TFC was measured by a previous method (Jia, Tang, & Wu, 1999) with some modifications. The sample solutions (10 mg/mL) were mixed with 50% ethanol and 5% sodium nitrite. After 1 h, 10% aluminum nitrate was added, and the mixtures were incubated for 6 min. The reaction was stopped by adding 1 M sodium hydroxide. The absorbance was immediately measured at 510 nm. The flavonoid contents were converted to milligram of

(±)-catechin equivalent (CE) per 100 gram of dry weight basis (mg CE/100 g dwb).

2.4.3. Vitamin C contents (VCC)

The VCC was determined photometrically after oxidation of ascorbic acid to dehydroascorbic acid, which reacts with 2,4-DNP to form a red complex (Mills, Daron, & Roe, 1949). The absorbance was measured at 520 nm, and contents were converted to milligram of L-ascorbic acid equivalent (AA) per 100 gram of dry weight basis (mg AA/100 g dwb).

2.4.4. Total carotenoid contents (TCC)

The TCC was determined by a previous method (Chandler & Schwartz, 1988) with some modifications. The method is based on saponification, followed by organic extraction whereafter total carotenoid contents in the extracts were measured using spectrophotometer at 450 nm. The TCC was converted to milligram of β-carotene equivalent per 100 gram of dry weight basis (mg/100 g dwb).

2.5. Polyphenolic compositions of Chinese cabbage leaves

The polyphenolic compositions were analyzed according to previous methods (Park, Hwang, Zheng, Kim, & Chung, 2010) with some modifications. After washing the chlorophyll from the leaves using 10 mL of chloroform, 1 g of sample powder of the Chinese cabbage leaves was extracted with 10 mL of 80% methanol at room temperature for 2 h and was filtered through a 0.45 μm filter for phenolic acids analysis. In order to acid hydrolysis for flavonoids analysis, these filtered solutions were evaporated to achieve dryness, and its dried extracts were 399.95 ± 0.33 mg. One hundred milligram of the dried extract was dissolved with 1 mL of 50% methanol. This dissolved solution was mixed with 9 mL of 2 M HCl in 50% methanol and was hydrolyzed at 90 °C for 4 h. After mixing 10 mL of ethyl acetate and vortexing, the supernatant was filtered and used as the sample solution for flavonoid analysis. The phenolic acid and flavonoid analyses were performed by using an HPLC (1260 Infinity Quaternary LC System, Agilent Technologies, Palo Alto, CA, USA) equipped with a 1024-element diode array detector (1260 Infinity Multiple Wavelength Detector VL, Agilent Technologies, Palo Alto, CA, USA) at 330 nm (C_{18} column, Develosil ODS-HG-5, 4.6 × 150 mm, Nomura Chemical Co., Aichi, Japan). The following binary mobile phases were used: A, 0.1% formic acid (v/v) in 40% methanol (v/v); and B, 0.1% formic acid (v/v) in acetonitrile: methanol (1:1, v/v). The gradient for HPLC analysis was linearly conducted for a total 20 min as follows: 100% A/0% B at zero min, 100% A/0% B at 2 min, 80% A/20% B at 5 min, 50% A/50% B at 10 min, and 50% A/50% B at 20 min. The flow rate was set to 0.4 mL/min with 35 °C of column temperature. Phenolic acids and flavonoids of the sample were confirmed by comparing their individual retention times with those of polyphenolic standards such as caffeic acid, sinapic acid, *p*-coumaric acid, ferulic acid, and myricetin. The identified phenolic acids and flavonoids were tentatively quantified (mg/100 g dwb) by the individual linear regression equation obtained from the standard calibration curve.

LC/MS analysis was conducted to identify the isolated phenolic acids and flavonoids. First, the polyphenolics of the sample were separated using an Agilent Technologies series 1200 system (Agilent Technologies, Palo Alto, CA, USA) equipped with an automatic degasser, quaternary pump, and auto-sampler. Chromatographic separations were performed on an ODS-HG-5 column (Develosil, 4.6 × 150 mm I.D). The column temperature was maintained at 35 °C, and the sample injection volume was 5 μL. The conditions of mobile phase and gradient were the same as that for HPLC analysis. Determination of molecular mass was performed with a 6410 Triple Quadrupole LC/MS system (Agilent Technologies, Palo Alto, CA, USA). Electro Spray Ionization (ESI)-MS was performed in the negative ion mode (600 °C, 20 psi). Data were collected using a mass scan from 0 to 460 *m*/*z*, at 1.5 s per scan.

2.6. Principal component analysis (PCA)

The results of the phenolic acid and flavonoid contents of the Chinese cabbage leaves were analyzed with PCA using the Statistical Analysis System (SAS, Version 9.3, SAS Institute Inc., Cary, NC, USA), which is a technique used for modeling a set of data onto itself (Abdi & Williams, 2010). Individual contents of the phenolic acids including caffeic acid, sinapic acid, *p*-coumaric acid, and ferulic acid and flavonoids including myricetin were selected as variables. In order to give all variables the same importance, the variables were "auto scaled", i.e., the average was subtracted from each variable, and each variable was divided by its standard deviation.

2.7. Statistical analysis

All of the examinations were executed in triplicate, and the values were expressed as the mean ± standard deviation. The results were evaluated through analysis of variance (ANOVA) with Duncan's multiple range tests ($p < 0.05$) using SAS.

3. Results and discussion

3.1. Antioxidant capacities of Chinese cabbage leaves

DPPH radical scavenging, FRAP, TEAC and ORAC assay were conducted to evaluate the antioxidant activities of three different types of Chinese cabbage leaves; the results are shown in Table 1. These methods are based on spectrophotometric methods and have been widely used to measure antioxidant activity in plant materials (Huang, Ou, & Prior, 2005). The DPPH (Blois, 1958), FRAP (Benzie & Strain, 1996), and TEAC (Miller, Rice-Evans, Davies, Gopinathan, & Milner, 1993) assays are based on electron transfer from the sample to the reagent radical and are measured spectrophotometrically by evaluating their color changes. The values of DPPH, FRAP, and TEAC were 92.17–239.17 μM TE, 87.00–714.50 μM TE, and 175.17–393.83 μM TE, respectively. The outer leaves (L1) had the strongest antioxidant capacity compared with the other leaves. The ORAC assay generally measures antioxidant inhibition of peroxyl radical ($ROO^{\cdot}$)-induced oxidations and thus reflects the classical radical chain-breaking antioxidant activity by H atom transfer (Ou et al., 2001). Traditional antioxidant assays followed extension of the lag phase only. However, antioxidant activities often extend well beyond the early stage of oxidation (Niki, 2002). To avoid inaccurate determination, the ORAC assay followed the reaction for extended periods (⩾60 min), and the antioxidant activity is calculated from the net integrated areas under the fluorescence decay curves (Prior, Wu, & Schaich, 2005). Therefore, this ORAC assay also has been broadly applied in various foods as a standard quantifying antioxidant assay since its development. The value of ORAC assay ranged from 493.37 μM TE to 1394.28 μM TE. The ORAC assay also showed the strongest activity in L1 and provided higher antioxidant activity values than other assays.

To support the results of antioxidant capacities of the Chinese cabbage leaves, the TPC, TFC, VCC and TCC were also determined; the results are shown in Table 1. The TPC was 148.81–347.46 mg GAE/100 g dwb. L1 had the highest amounts of total phenolics, which is consistent with previous research on the TPC of Brassicaceae (Heimler, Vignolini, Dini, Vincieri, & Romani, 2006). The

Table 1
Antioxidant activities and contents of Chinese cabbage leaves.

	Antioxidant activities (μM TE)				Antioxidant contents (mg/100 g dwb)			
	DPPH	FRAP	TEAC	ORAC	TPC	TFC	VCC	TCC
L1	239.17 ± 16.80[a]	714.50 ± 154.58[a]	393.83 ± 27.30[a]	1394.28 ± 7.06[a]	347.46 ± 32.17[a]	328.17 ± 17.07[a]	13.67 ± 0.54[a]	18.87 ± 0.01[a]
L2	106.17 ± 6.51[b]	102.83 ± 11.81[b]	175.17 ± 28.75[b]	562.51 ± 48.77[b]	150.06 ± 5.02[b]	75.37 ± 12.34[b]	9.84 ± 1.21[b]	14.37 ± 0.01[b]
L3	92.17 ± 7.64[b]	87.00 ± 6.61[b]	232.50 ± 61.02[b]	493.37 ± 20.95[c]	148.81 ± 5.68[b]	61.29 ± 10.68[b]	7.04 ± 0.50[c]	3.93 ± 0.01[c]

L1, outer leaves; L2, mid-leaves; L3, inner leaves; DPPH, α,α-diphenyl-2-picrylhydrazyl radical scavenging activity; FRAP, ferric ion reducing antioxidant power; TEAC, trolox equivalent antioxidant capacity; ORAC, oxygen radical absorbance capacity; TPC, total phenolic contents; TFC, total flavonoid contents; VCC, vitamin C contents; TCC, total carotenoid contents.
The values of TPC, TFC and TCC were expressed to gallic acid, catechin and β-carotene equivalent, respectively.
[a,b,c]Data are shown as mean ± SD, $n = 3$. Data with different superscript letters in the same column are significantly different ($p < 0.05$).

TFC was 61.29–328.17 mg CE/100 g dwb. Flavonoids are secondary metabolites and have several biological activities such as antioxidant, anti-inflammatory, anti-microbial, and anti-cancer activities (Middleton, Kandaswami, & Theoharides, 2000). Myricetin, quercetin, and kaempferol are major flavonoids in Brassicaceae including Chinese cabbage (Formica & Regelson, 1995; Miean & Mohamed, 2001). Thus, the TFC values in this study were also high. The vitamin C contents ranged from 7.04 mg/100 g to 13.67 mg/100 g, which is consistent with the trends of TPC and TFC. L-Ascorbic acid, known as vitamin C, plays an important role in collagen biosynthesis and iron absorption (Mandl, Szarka, & Banhegyi, 2009). The TCC ranged from 3.93 mg/100 g to 18.87 mg/100 g, which is consistent with the trends of other antioxidants. Carotenoids are precursor of vitamin A, and it plays an important role on the skin, skeleton tissues and respiratory organs (Akter, Ahmed, & Eun, 2010). These antioxidant compounds including phenolics, flavonoids, vitamin C, and carotenoids are biosynthesized when the plant is exposed to environmental stress (Kusznierewicz et al., 2008). Chlorophylls are also a kind of plant phytochemicals with antioxidant activity at high level (Cervantes-Paz et al., 2014; Ferruzzi, Böhm, Courtney, & Schwartz, 2002), although they were not taken into account in this study because of the limitations of the spectroscopic methods for antioxidant activities and contents. In general, the concentration of phytochemicals such as polyphenolics varied according to cultivar, growing area, and harvest time (Kang, Ibrahim, Juvik, Kim, & Kang, 2006; Wu et al., 2008). Moreover, they play a critical role in the protection of plants against UV radiation (Gitz, Liu, & McClure, 1998). The high levels of phytochemicals are also associated with increased resistance to fungal plant pathogens (Nicholson & Hammerschmidt, 1992). The longer exposure to sunlight during the cultivation might cause the outer leaves of Chinese cabbage to contain more antioxidants than those in the inner leaves as well (Ji et al., 2011).

3.2. Polyphenolic composition of Chinese cabbage leaves

The polyphenolics in plants include two main types of compounds: phenolic acids and flavonoids. The phenolic acids are compounds with at least one aromatic ring bearing one or more hydroxyl groups (Hounsome, Hounsome, Tomos, & Edwards-Jones, 2008). Hydroxycinnamic acid derivatives such as sinapic acid, caffeic acid, ferulic acid, and *p*-coumaric acid are found in lettuce, tomatoes, pac choi, and cabbage (Miean & Mohamed, 2001). They have strong antioxidant activities due to scavenging ROS (Cheng, Dai, Zhou, Yang, & Liu, 2007) and inhibition of lipid oxidation (Sroka & Cisowski, 2003). Flavonoids such as myricetin present in Chinese cabbage possess anti-inflammatory, antiviral, and antioxidant properties (Heim, Tagliaferro, & Bobilya, 2002). In this study, the polyphenolic contents in the Chinese cabbage leaves were analyzed by HPLC. Caffeic acid, sinapic acid, *p*-coumaric acid, and ferulic acid as phenolic acids were isolated from the Chinese cabbage leaves (Fig. 2A). After acid hydrolysis, myricetin as a flavonoid was also isolated (Fig. 2B). Flavonoids such as myricetin in plant mostly present as glycosides. These glycosides can be hydrolyzed and absorbed in the human stomach by strong acid. Likewise these flavonoids could be analyzed by HPLC through acid hydrolysis. These phenolic acids and the flavonoid were also identified by LC/MS analysis (Table 2); the *m*/*z* (mass to charge ratio) values of LC/MS data were 179.0, 223.1, 163.0, 193.1, and 317.1 $[M-H]^-$, successfully matched with the molecular weights of caffeic acid, sinapic acid, *p*-coumaric acid, ferulic acid, and myricetin, respectively.

The isolated phenolic acid and flavonoid contents are shown in Table 3. A high amount of sinapic acid, the major phenolic acid in Chinese cabbage (Jiang et al., 2013; Mattila & Hellström, 2007), was observed in all three leaf types at 6.01–8.00 mg/100 g, as were *p*-coumaric acid (2.20–2.89 mg/100 g) and myricetin (0.80–0.83 mg/100 g). In contrast, caffeic acid (1.39 mg/100 g) and ferulic acid (0.47 mg/100 g) were contained in L1. Similar to that for the high antioxidant contents, the longer exposure time of L1 to sunlight might be the cause of the varied distributions of the polyphenolics.

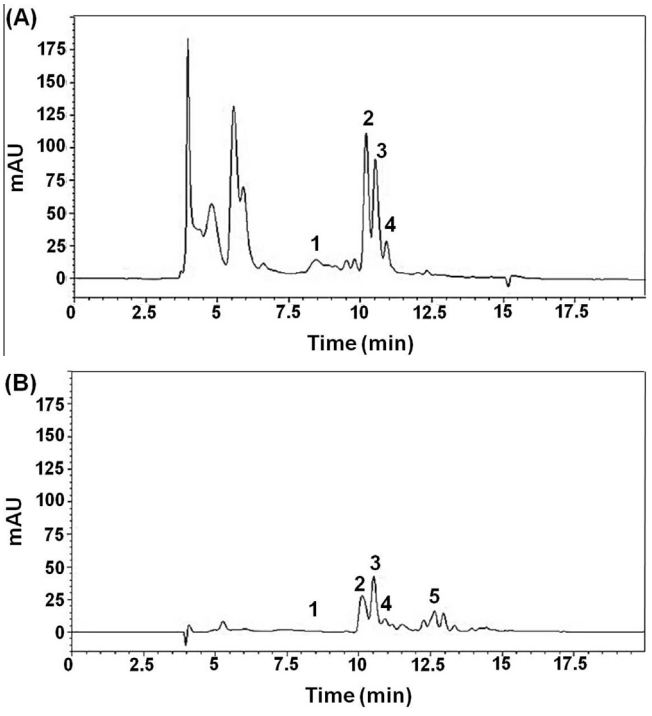

Fig. 2. High performance liquid chromatography (HPLC) chromatograms of (A) phenolic acid and (B) flavonoid analyses in the outer leaves (L1) of Chinese cabbage. 1, caffeic acid; 2, sinapic acid; 3, *p*-coumaric acid; 4, ferulic acid; 5, myricetin.

Table 2
Identification of phenolic acids and flavonoids in the Chinese cabbage leaves by high performance liquid chromatography (HPLC) and liquid chromatography/mass spectrometry (LC/MS) analysis.

Compounds		Rt (min)	$[M-H]^-$
Phenolic acids	Caffeic acid	8.5	179.0
	Sinapic acid	10.2	223.1
	p-Coumaric acid	10.5	163.0
	Ferulic acid	10.9	193.1
Flavonoids	Myricetin	12.6	317.1

Rt, retention time.

Table 3
Phenolic acid and flavonoid contents of Chinese cabbage leaves.

Polyphenolics (mg/100 g dwb)	L1	L2	L3
Caffeic acid	1.39 ± 0.06[a]	ND	ND
Sinapic acid	6.82 ± 0.46[b]	6.01 ± 0.43[c]	8.00 ± 0.08[a]
p-Coumaric acid	2.89 ± 0.17[a]	2.24 ± 0.17[b]	2.20 ± 0.07[b]
Ferulic acid	0.47 ± 0.05[a]	ND	ND
Myricetin	0.83 ± 0.01[a]	0.80 ± 0.00[b]	0.82 ± 0.00[a]
Total	12.40 ± 0.75[a]	9.05 ± 0.60[c]	11.02 ± 0.15[b]

The values were determined at the mg/100 g dwb (dry weight basis) level.
L1, outer leaves; L2, mid-leaves; L3, inner leaves.
ND, not detected.
[a,b,c]Data are shown as mean ± SD, $n = 3$. Data with same superscript letters in the same row are not significantly different ($p < 0.05$).

3.3. PCA of polyphenolics in Chinese cabbage leaves

PCA allows for easy visualization of complex data according to the similarity of the grouped data. It is closely related to the method of distinguishing and determining a compound showing large-scale dispersion in a population. Therefore, PCA is a powerful tool for pattern recognition, classification, modeling, and other aspects of data evaluation without preliminary grouping (Sousa et al., 2008). In this study, PCA was applied to assess the relationships between outer, mid-, and inner Chinese cabbage leaves depending on the phenolic acids and flavonoid contents. The contents of caffeic acid, sinapic acid, *p*-coumaric acid, ferulic acid, and myricetin were subjected to PCA to outline the differences in the Chinese cabbage leaves. The results were indicated by plotting the principal component (PC) scores. In PCA, the PC is extracted, which explains the largest amount of variation among the three leaf types. A second PC is then derived to account for the maximum variation in the remaining unexplained variability. Each PC is a linear combination of the original variables. The relationship between the original variables and the samples can be observed by their projection on these few PCs (Lee, Kim, Kim, Chang, & Lee, 2012). In Fig. 3, the abscissa represents a PC 1 score, whereas the ordinate represents a PC 2 score. The results indicate that the all of the eigenvalues of the two principal components were higher than 1.0 and accounted for more than 95.04% of the total variance. PC 1 and PC 2 explained 74.93% and 20.11% of the total variance, respectively. In the PCA plot with two components, three distinct groups were identifiable (Fig. 3A). The three leaf types, L1, L2 and L3, were clearly distinguished through PCA. In Fig. 3B, the corresponding loading in PC 1 was positive for caffeic acid, *p*-coumaric acid, ferulic acid, and myricetin, but it was negative for sinapic acid. In PC 2, it was positive for all phenolic acids, except myricetin, the flavonoid. Moreover, caffeic acid, *p*-coumaric acid, ferulic acid, and myricetin were grouped, whereas sinapic acid was separated from other phenolics. Thus, the outer and inner leaves differ mainly because of their sinapic acid contents. These PCA results also showed that the proper score range of the principal components could be used for outstanding sample distinction depending on the correlation between the three variables and these two PCs.

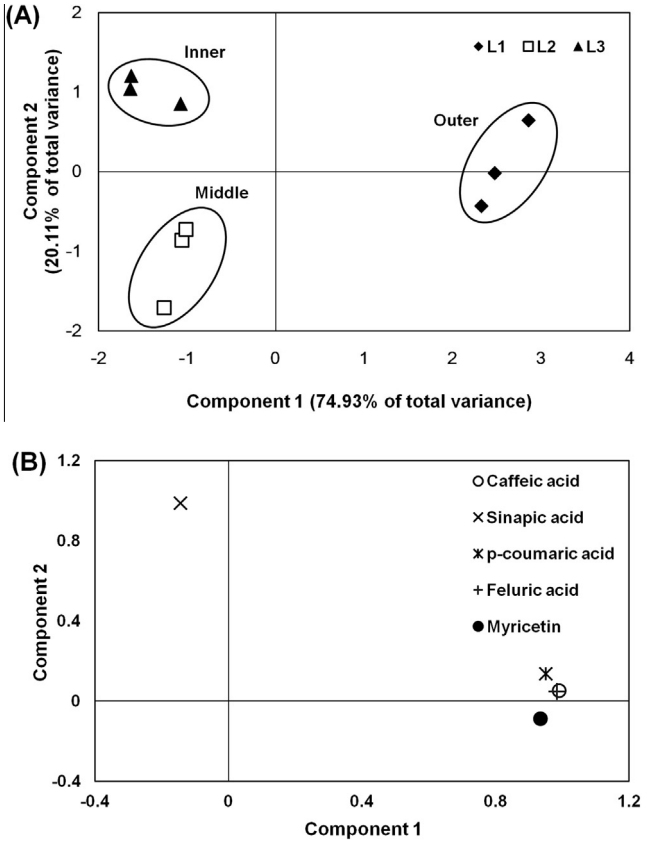

Fig. 3. (A) Scores and (B) loading plots of principal component (PC) 1 and PC 2 of the PC analysis (PCA) results obtained from the phenolic acid and flavonoid data of the three sections from inner to outer leaves.

4. Conclusion

In this study, Chinese cabbage leaves were divided into three types to investigate their different antioxidant capacities and polyphenolics. The research accomplished confirmed a significant diversity in the polyphenolic contents and in the antioxidant activities related to them. The phenolic acids and flavonoid profiles are characteristic for the particular part of Chinese cabbage leaves. In all determinations of antioxidant activities and antioxidant contents, the outer leaves had the highest levels, followed by the mid- and inner leaves (L1 > L2 > L3). These results reveal that the trend of antioxidant contents is the same as the order of the antioxidant activities. In HPLC and LC/MS analysis, the four kinds of phenolic acids (caffeic acid, sinapic acid, *p*-coumaric acid and ferulic acid) and one flavonoid (myricetin) were identified and were quantified by comparison with polyphenolics standards and linear regression equations. Sinapic acid showed the highest contents in all leaf types; *p*-coumaric acid and myricetin also were contained in all leaves. On the contrary, caffeic acid and ferulic acid were contained in only the outer portion. Thus, it can be assumed that longer exposure time to sunlight leads to high antioxidant contents and production of varied antioxidants in Chinese cabbage. On the other hand, the outer leaves can contribute to improving health value. The PCA identified the contribution of the PC in the Chinese cabbage. The Chinese cabbage leaves showed significant statistical differences according to the different leaf types in the PCA. Antioxidant capacities and sinapic acid are important indicators that can be distinguished between outer and inner leaves. It could be used in chemotaxonomic classification and confirmation of authenticity. Thus, this study can be very helpful for the

development of new fresh-cut products using each particular part depending on the purpose of consumption.

Acknowledgments

This research was supported by the Advanced Production Technology Development Program of the Ministry of Agricultural, Food, and Rural Affairs of Republic of Korea.

References

Abdi, H., & Williams, L. J. (2010). Principal component analysis. *Wiley Interdisciplinary Reviews: Computational Statistics, 2*(4), 433–459. http://dx.doi.org/10.1002/wics.101.

Akter, M. S., Ahmed, M., & Eun, J. B. (2010). Effect of blanching and drying temperatures on the physicochemical characteristics, dietary fiber composition and antioxidant-related parameters of dried persimmons peel powder. *International Journal of Food Sciences and Nutrition, 61*(7), 702–712. http://dx.doi.org/10.3109/09637481003757852.

Amin, I., & Lee, W. Y. (2005). Effect of different blanching times on antioxidant properties in selected cruciferous vegetables. *Journal of the Science of Food and Agriculture, 85*(13), 2314–2320. http://dx.doi.org/10.1002/jsfa.2261.

Benzie, I. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. *Analytical Biochemistry, 239*(1), 70–76. http://dx.doi.org/10.1006/abio.1996.0292.

Blois, M. (1958). Antioxidant determinations by the use of a stable free radical. *Nature, 181*, 1199–1200 (Retrieved from: <http://www.nature.com/nature/journal/v181/n4617/abs/1811199a0.html>).

Cervantes-Paz, B., Yahia, E. M., de Jesús Ornelas-Paz, J., Victoria-Campos, C. I., Ibarra-Junquera, V., Pérez-Martínez, J. D., & Escalante-Minakata, P. (2014). Antioxidant activity and content of chlorophylls and carotenoids in raw and heat-processed Jalapeño peppers at intermediate stages of ripening. *Food Chemistry, 146*, 188–196. http://dx.doi.org/10.1016/j.foodchem.2013.09.060.

Chandler, L. A., & Schwartz, S. J. (1988). Isomerization and losses of trans-β-carotene in sweet potatoes as affected by processing treatments. *Journal of Agricultural and Food Chemistry, 36*(1), 129–133. http://dx.doi.org/10.1021/jf00079a033.

Cheng, J., Dai, F., Zhou, B., Yang, L., & Liu, Z. (2007). Antioxidant activity of hydroxycinnamic acid derivatives in human low density lipoprotein: Mechanism and structure–activity relationship. *Food Chemistry, 104*(1), 132–139 (Retrieved from: <http://www.sciencedirect.com/science/article/pii/S0308814606008764>).

Ferruzzi, M. G., Böhm, V., Courtney, P. D., & Schwartz, S. J. (2002). Antioxidant and antimutagenic activity of dietary chlorophyll derivatives determined by radical scavenging and bacterial reverse mutagenesis assays. *Journal of Food Science, 67*(7), 2589–2595. http://dx.doi.org/10.1111/j.1365-2621.2002.tb08782.x.

Formica, J. V., & Regelson, W. (1995). Review of the biology of Quercetin and related bioflavonoids. *Food and Chemical Toxicology: An International Journal Published for the British Industrial Biological Research Association, 33*(12), 1061–1080.

Gitz, D. C., Liu, L., & McClure, J. W. (1998). Phenolic metabolism, growth, and UV-b tolerance in phenylalanine ammonia-lyase-inhibited red cabbage seedlings. *Phytochemistry, 49*(2), 377–386. http://dx.doi.org/10.1016/S0031-9422(98)00011-9.

Harbaum, B., Hubbermann, E. M., Wolff, C., Herges, R., Zhu, Z., & Schwarz, K. (2007). Identification of flavonoids and hydroxycinnamic acids in pak choi varieties (*Brassica campestris* L. ssp. chinensis var. communis) by HPLC–ESI-MS*n* and NMR and their quantification by HPLC-DAD. *Journal of Agricultural and Food Chemistry, 55*(20), 8251–8260. http://dx.doi.org/10.1021/jf071314+.

Heim, K. E., Tagliaferro, A. R., & Bobilya, D. J. (2002). Flavonoid antioxidants: Chemistry, metabolism and structure–activity relationships. *The Journal of Nutritional Biochemistry, 13*(10), 572–584.

Heimler, D., Vignolini, P., Dini, M. G., Vincieri, F. F., & Romani, A. (2006). Antiradical activity and polyphenol composition of local Brassicaceae edible varieties. *Food Chemistry, 99*(3), 464–469. http://dx.doi.org/10.1016/j.foodchem.2005.07.057.

Hounsome, N., Hounsome, B., Tomos, D., & Edwards-Jones, G. (2008). Plant metabolites and nutritional quality of vegetables. *Journal of Food Science, 73*(4), R48–R65. http://dx.doi.org/10.1111/j.1750-3841.2008.00716.x.

Huang, D., Ou, B., & Prior, R. L. (2005). The chemistry behind antioxidant capacity assays. *Journal of Agricultural and Food Chemistry, 53*(6), 1841–1856. http://dx.doi.org/10.1021/jf030723c.

Huxley, R. R., & Neil, H. A. W. (2003). The relation between dietary flavonol intake and coronary heart disease mortality: A meta-analysis of prospective cohort studies. *European Journal of Clinical Nutrition, 57*(8), 904–908. http://dx.doi.org/10.1038/sj.ejcn.1601624.

Isabelle, M., Lee, B. L., Lim, M. T., Koh, W. P., Huang, D., & Ong, C. N. (2010). Antioxidant activity and profiles of common vegetables in Singapore. *Food Chemistry, 120*(4), 993–1003. http://dx.doi.org/10.1016/j.foodchem.2009.11.038.

Islam, M. S., & Choi, H. (2008). Chinese cabbage (*Brassica campestris* L.) does not improve glucose tolerance, serum insulin, or blood lipid profiles in a rat model of type-2 diabetes. *Journal of Food Science, 73*(9), H213–H217. http://dx.doi.org/10.1111/j.1750-3841.2008.00958.x.

Ji, L., Wu, J., Gao, W., Wei, J., Yang, J., & Guo, C. (2011). Antioxidant capacity of different fractions of vegetables and correlation with the contents of ascorbic acid, phenolics, and flavonoids. *Journal of Food Science, 76*(9), C1257–C1261. http://dx.doi.org/10.1111/j.1750-3841.2011.02402.x.

Jia, Z., Tang, M., & Wu, J. (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chemistry, 64*(4), 555–559. http://dx.doi.org/10.1016/S0308-8146(98)00102-2.

Jiang, N., Chung, S. O., Lee, J., Ryu, D., Lim, Y. P., Park, S., & An, G. (2013). Increase of phenolic compounds in new Chinese cabbage cultivar with red phenotype. *Horticulture, Environment, and Biotechnology, 54*(1), 82–88. http://dx.doi.org/10.1007/s13580-013-0136-5.

Kang, J. Y., Ibrahim, K. E., Juvik, J. A., Kim, D. H., & Kang, W. J. (2006). Genetic and environmental variation of glucosinolate content in Chinese cabbage. *HortScience, 41*(6), 1382–1385 (Retrieved from: <http://hortsci.ashspublications.org/content/41/6/1382.short>).

Kim, B. S., & Klieber, A. (1997). Quality maintenance of minimally processed Chinese cabbage with low temperature and citric acid dip. *Journal of the Science of Food and Agriculture, 75*(1), 31–36. http://dx.doi.org/10.1002/(SICI)1097-0010(199709)75:1<31::AID-JSFA836>3.0.CO;2-X.

Kim, D. O., Padilla-Zakour, O. I., & Griffiths, P. D. (2006). Flavonoids and antioxidant capacity of various cabbage genotypes at juvenile stage. *Journal of Food Science, 69*(9), C685–C689. http://dx.doi.org/10.1111/j.1365-2621.2004.tb09916.x.

Kusznierewicz, B., Bartoszek, A., Wolska, L., Drzewiecki, J., Gorinstein, S., & Namieśnik, J. (2008). Partial characterization of white cabbages (*Brassica oleracea* var. capitata f. alba) from different regions by glucosinolates, bioactive compounds, total antioxidant activities and proteins. *LWT – Food Science and Technology, 41*(1), 1–9. http://dx.doi.org/10.1016/j.lwt.2007.02.007.

Lee, K. S., Kim, G. H., Kim, H. H., Chang, Y. I., & Lee, G. H. (2012). Volatile compounds of Panax ginseng C.A. Meyer cultured with different cultivation methods. *Journal of Food Science, 77*(7), C805–C810. http://dx.doi.org/10.1111/j.1750-3841.2012.02765.x.

Lim, Y. Y., Lim, T. T., & Tee, J. J. (2007). Antioxidant properties of several tropical fruits: A comparative study. *Food Chemistry, 103*(3), 1003–1008. http://dx.doi.org/10.1016/j.foodchem.2006.08.038.

Mandl, J., Szarka, A., & Banhegyi, G. (2009). Vitamin C: Update on physiology and pharmacology. *British Journal of Pharmacology, 157*(7), 1097–1110. http://dx.doi.org/10.1111/j.1476-5381.2009.00282.x.

Mattila, P., & Hellström, J. (2007). Phenolic acids in potatoes, vegetables, and some of their products. *Journal of Food Composition and Analysis, 20*(3), 152–160 (Retrieved from: <http://www.sciencedirect.com/science/article/pii/S0889157506001050>).

Middleton, E., Kandaswami, C., & Theoharides, T. C. (2000). The effects of plant flavonoids on mammalian cells: Implications for inflammation, heart disease, and cancer. *Pharmacological Reviews, 52*(4), 673–751 (Retrieved from: <http://www.ncbi.nlm.nih.gov/pubmed/11121513>).

Miean, K. H., & Mohamed, S. (2001). Flavonoid (myricetin, quercetin, kaempferol, luteolin, and apigenin) content of edible tropical plants. *Journal of Agricultural and Food Chemistry, 49*(6), 3106–3112.

Miller, N. J., Rice-Evans, C., Davies, M. J., Gopinathan, V., & Milner, A. (1993). A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. *Clinical Science (London, England: 1979), 84*(4), 407–412 (Retrieved from: <http://www.ncbi.nlm.nih.gov/pubmed/8482045>).

Mills, M. B., Damron, C. M., & Roe, J. H. (1949). Ascorbic acid, dehydroascorbic acid, and diketogulonic acid in fresh and processed foods. *Analytical Chemistry, 21*(6), 707–709. http://dx.doi.org/10.1021/ac60030a019.

Nicholson, R. L., & Hammerschmidt, R. (1992). Phenolic compounds and their role in disease resistance. *Annual Review of Phytopathology, 30*(1), 369–389. http://dx.doi.org/10.1146/annurev.py.30.090192.002101.

Niki, E. (2002). Antioxidant activity: Are we measuring it correctly? *Nutrition (Burbank, Los Angeles County, Calif.), 18*(6), 524–525 (Retrieved from: <http://www.ncbi.nlm.nih.gov/pubmed/12044829>).

Oboh, G., Raddatz, H., & Henle, T. (2008). Antioxidant properties of polar and non-polar extracts of some tropical green leafy vegetables. *Journal of the Science of Food and Agriculture, 88*(14), 2486–2492. http://dx.doi.org/10.1002/jsfa.3367.

Ou, B., Hampsch-Woodill, M., & Prior, R. L. (2001). Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe. *Journal of Agricultural and Food Chemistry, 49*(10), 4619–4626 (Retrieved from: <http://www.ncbi.nlm.nih.gov/pubmed/11599998>).

Park, J. S., Hwang, I. W., Zheng, H. Z., Kim, S. K., & Chung, S. K. (2010). Determination of optimum hydrolysis conditions for flavonoid analysis in plant leaves. *Korean Journal of Food Preservation, 17*(2), 261–266 (Retrieved from : <http://db.koreascholar.com/article.aspx?code=41797>).

Prior, R. L., Wu, X., & Schaich, K. (2005). Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agricultural and Food Chemistry, 53*(10), 4290–4302. http://dx.doi.org/10.1021/jf0502698.

Roy, M., Takenaka, M., Isobe, S., & Tsushida, T. (2007). Antioxidant potential, anti-proliferative activities, and phenolic content in water-soluble fractions of some commonly consumed vegetables: Effects of thermal treatment. *Food Chemistry, 103*(1), 106–114 (Retrieved from: <http://www.sciencedirect.com/science/article/pii/S0308814606006182>).

Sariburun, E., Sahin, S., Demir, C., Türkben, C., & Uylaşer, V. (2010). Phenolic content and antioxidant activity of raspberry and blackberry cultivars. *Journal of Food Science, 75*(4), C328–C335. http://dx.doi.org/10.1111/j.1750-3841.2010.01571.x.

Singleton, V. L., Orthofer, R., & Lamuela-Raventos, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Methods in enzymology, 299*, 152–178. http://dx.doi.org/10.1016/S0076-6879(99)99017-1.

Sousa, C., Pereira, D. M., Pereira, J. A., Bento, A., Rodrigues, M. A., Dopico-García, S., & Andrade, P. B. (2008). Multivariate analysis of tronchuda cabbage (*Brassica oleracea* L. var. costata DC) phenolics: Influence of fertilizers. *Journal of Agricultural and Food Chemistry, 56*(6), 2231–2239. http://dx.doi.org/10.1021/jf073041o.

Sroka, Z., & Cisowski, W. (2003). Hydrogen peroxide scavenging, antioxidant and anti-radical activity of some phenolic acids. *Food and Chemical Toxicology: An International Journal Published for the British Industrial Biological Research Association, 41*(6), 753–758 (Retrieved from: <http://www.ncbi.nlm.nih.gov/pubmed/12738180>).

Subhasree, B., Baskar, R., Laxmi Keerthana, R., Lijina Susan, R., & Rajasekaran, P. (2009). Evaluation of antioxidant potential in selected green leafy vegetables. *Food Chemistry, 115*(4), 1213–1220. http://dx.doi.org/10.1016/j.foodchem.2009.01.029.

Watanabe, M., Musumi, K., & Ayugase, J. (2011). Carotenoid pigment composition, polyphenol content, and antioxidant activities of extracts from orange-colored Chinese cabbage. *LWT – Food Science and Technology, 44*(9), 1971–1975. http://dx.doi.org/10.1016/j.lwt.2011.04.010.

Wu, J., Yuan, Y. X., Zhang, X. W., Zhao, J., Song, X., Li, Y., & Wang, X. W. (2008). Mapping QTLs for mineral accumulation and shoot dry biomass under different Zn nutritional conditions in Chinese cabbage (*Brassica rapa* L. ssp. pekinensis). *Plant and Soil, 310*(1–2), 25–40. http://dx.doi.org/10.1007/s11104-008-9625-1.