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Effects of Soil pH on Nutritional and Functional Components of Chinese Cabbage (*Brassica rapa* ssp. *campestris*)

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Abstract. The contents of functional and nutritional components of 13 cultivars of Chinese cabbage (CC, *Brassica rapa* subspecies *campestris*) were analyzed to compare the effects of soil pH of the greenhouse (pH 6.2) and outdoor (pH 7.6). The CC cultivated on pH 6.2 (CC-6.2) soil contained significantly increased amounts (2-9 fold) of pectin, crude protein, vitamin C and vitamin E compared to the counterpart (CC-7.6). The contents of ash and the minerals (Ca, Fe, Na, and Mn) were also significantly increased in CC-6.2. However, CC-6.2 contained 40-50% lower contents of reducing sugars, cellulose and crude fat than CC-7.6. CC-7.6 contained more glucosinolates, gluconasturtiin (18.33 vs. 1.16 $nmol \cdot g^{-1}$ wet weight) and gluconapin (145 vs. 2 $nmol \cdot g^{-1}$ wet wt), than CC-6.2. In conclusion, CC-6.2 had an improved texture (high pectin and low cellulose) and nutritional value (high in protein, Ca, Fe, vitamin C, and E), whereas the CC-7.6 had better taste (high in reducing sugars) and anticancer functionality (high in glucosinolates).

Additional key words**:** dietary fiber, glucosinolate, mineral, vitamin

Introduction

Chinese cabbage (CC; *Brassica rapa* ssp. *campestris*) is one of the most important Brassica crops, with a world-wide production in 2007 of 53 million tons (http://www.kosaseed.or.kr). This crop is extremely important in Korea, being the major component of Kimchi, a staple vegetable of the Korean diet. About 2.25 million tons of CC is produced annually in Korea (Crop statistics, 2007, Ministry of Agriculture, Fishery, and Food of Korea) and about 90 g/day of Kimchi is consumed by the average adult (Nan et al., 2005).

Dietary fibers, including pectin and cellulose, are beneficial to human health and supplied only from plants (Wardlaw, 1999). Reducing sugars, including glucose, affect the quality of vegetables because they provide a sweet taste. Protein and fat affect not only the taste of vegetables but also nutritional values. Vitamins C and E have antioxidant and anticancer properties, and are supplied mainly from vegetables (Wardlaw, 1999). Cruciferous plants, including CC, are important sources for anticancer “nutraceutical” compounds, e.g., β-carotene, vitamin C, fibers (including pectin and cellulose), calcium, lutein, and zeaxanthin (Divisi et al., 2006), glucosinolates (Hayes et al., 2008), and phenolics (Harbaum et al., 2007). Calcium and iron are often used in dietary supplements for women, and they are also available from leafy vegetables (Wardlaw, 1999). In this study, the moisture levels and contents of reducing sugar, pectin, cellulose, protein, fat, vitamin C, vitamin E, glucosinolates, Ca and other minerals in CC were measured for the determination of CC quality.

Many factors including soil, weather, fertilization and others can affect the chemical composition of plants (Tahvonen, 1993). Certain cultivars of CC are often cultivated in greenhouses during cold weather periods. Since the soil in a greenhouse is rarely exposed to rain and is repeatedly supplemented with fertilizers (e.g. ammonium sulfate), it can become acidic by the accumulation of N and S after the repeated cultivation of crops. To prevent soil acidification and to supply Ca, farmers often apply $Ca(OH)_2$ or $CaCO_3$.

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The repeated cultivation of CC in greenhouses definitely affects the quality and composition of the plant.

For the cultivation of vegetables, the proper range of soil pH is 6.0-6.5. If there is a severe accumulation of salts, the soil pH may be over pH 7.0. This is usually due to the close system that cause accumulation of phosphate and nitrate (5- to 10-fold accumulation than normal soil) which increases osmotic pressure and quantity of ammonia, and thus $Ca(OH)_2$ is hardly absorbed by plants. In case of Chinese cabbage, if the soil pH is >7, growth is poor and rotten inside because of lack of Ca. This also accelerated by insolublized Mn or B due to a high pH (Ryu et al., 2006).

The pH of some land may be higher than the optimal one (>pH 6.5), affecting the quality of CC. To date, there have been no reports on the effects of soil pH on the nutritional components of CC. In this study, 13 cultivars were tested to distinguish the effects of cultivar specificity from those of the soil pH, thus providing information useful to food scientists and plant breeders.

Table 1. The 13 Chinese cabbage cultivars (*Brassica rapa* ssp. *campestris*) used in this study.

Cultivar	Access number[z]
Fuxing 80	26139
Jinguan 80 (2dai)	26142
Liaobaishihao	26145
Luixing 70	26146
Tianjinlui qingmaye	26148
Jincaisanhao	26151
Xingchengmaye	26152
Tianxiawushuang	26153
Dongbao	26156
Qioufuyidaijiaopei	26161
Fengying	26164
Qishitianfabaicai	26168
Fubaierhao	26169

[z] Access number in Korea Brassica Genome Resource Bank (Daejeon, Korea).

Materials and Methods

Plant materials and sample preparation

Thirteen cultivars of *B. rapa* originating from China were obtained from the Korea Brassica Genome Resource Bank (Daejeon, Korea) (Table 1). They were cultivated on neutral soil in a side-open greenhouse and on acidified soil at Chungnam National University Farm, Daejeon, Korea, from August to October of 2007; the two farms are about 40 km apart. The data from soil analyses at each farm are shown in Table 2. The two groups were sampled after confirmation of uniformity in color, shape, and size of the mature plants, based on criteria given by the Korea Seed & Variety Service. For each trial, a cultivar was grown in eight replicates, and three plants were randomly sampled. For glucosinolates and minerals, only one replication was used.

The third-layer outer leaves were used for component analyses. The sample leaves were mixed with two volumes of distilled water and homogenized with a blender. The blended samples were kept at -70℃. Before use, the blended samples were homogenized with a Polytron® (PT-MR 2100, Kinematica AG, Switzerland). For component analyses using unblended samples, the sample leaves were kept at 4℃ and used within 3 days.

Analysis for components

Water and ash; Water contents were measured by the Infrared Moisture Determination Balance (FD-240, Kett Electric

Table 2. Minerals in the soils of two different pHs for Chinese cabbage cultivation.

Mineral	Soil dissolved in 0.1N HCl pH 6.2 (mg·g^{-1} wet wt)	Soil dissolved in 0.1N HCl pH 7.6 (mg·g^{-1} wet wt)	Ratio	Soil dissolved in distilled water pH 6.2 (mg·g^{-1} wet wt)	Soil dissolved in distilled water pH 7.6 (mg·g^{-1} wet wt)	Ratio
Ca	2.5 ± 0.3	1.5 ± 0.4	1.7	0.8 ± 0.4	0.9 ± 0.2	1.1
Mg	0.6 ± 0.0	0.4 ± 0.1	1.5	0.2 ± 0.1	0.2 ± 0.1	0.6
Fe	0.4 ± 0.0	0.2 ± 0.0	3.2	ND	0.1 ± 0.0	-
K	0.2 ± 0.0	0.2 ± 0.0	0.6	0.1 ± 0.0	0.3 ± 0.1	1.0
Mn	0.1 ± 0.0	0.1 ± 0.0	0.8	0.0 ± 0.0	0.0 ± 0.0	0.7
Na	0.1 ± 0.0	0.0 ± 0.0	3.3	0.1 ± 0.0	0.0 ± 0.0	2.3
Zn	0.0 ± 0.0	0.0 ± 0.0	3.9	0.0 ± 0.0	0.0 ± 0.0	1.0
B	0.0 ± 0.0	ND	-	0.0 ± 0.0	0.0 ± 0.1	0.3
S	0.4 ± 0.2	0.0 ± 0.0	11.7	0.4 ± 0.2	0.0 ± 0.0	36.6
P	0.2 ± 0.0	0.0 ± 0.0	9.2	0.0 ± 0.0	0.0 ± 0.0	0.4
pH				6.2 ± 0.3	7.6 ± 0.4	

[ND] Not detected.

Laboratory, Japan). The dried samples used for moisture content were also used for measuring ash contents by furnace combustion at 550℃ for 3-6 hours (AOAC, 1995).

Minerals; Fresh leaves (10 g) were digested by mixing with 40 mL of nitric acid (60-62%) and 10 mL of hydrogen peroxide (30%) on a heating plate with stirring for 1 h (AOAC, 1995). The minerals in the digested samples were measured by Inductively Coupled Plasma Atomic Emission Spectrometer (Optima 3300DV, Perkin-Elmer Instruments, Shelton, USA). For soil analyses, soil was dissolved in 10 volumes (w/v) of distilled water or 0.1N HCl. After centrifugation (1,000 x g, 5 min), the supernatants were used. The soil pH was measured with the supernatant obtained from distilled water (Table 2).

Reducing sugar; The reducing sugar contents were measured by the dinitrosalicylic acid (DNS) method (Miller, 1959). For DNS solution, 5 g of 3,5-dinitrosalicylic acid was dissolved in 100 mL of 2 N NaOH with heating and then 150 g of Rochell salt (potassium sodium tartrate tetrahydrate) were added along with sufficient distilled water to bring the final volume to 500 mL. The homogenized samples (1 mL) were centrifuged (22,300 × g, 3 min) and the supernatant was diluted. DNS solution (300 μL) and the diluted sample (300 μL) were mixed and incubated for 15 min at 100℃. After cooling, the sample solution was further diluted and the absorbance at 540 nm was measured by the microplate reader (E Max, Molecular Devices, Sunnyvale, CA). Glucose was used as the standard.

Pectin; The water-insoluble pectin contents were measured by the method of Manabe (Manabe and Naohara, 1986). The homogenized samples (1 mL) were centrifuged and the precipitates were washed twice with 95% (v/v) ethanol. The precipitates were further washed with acetone and then dried for 48 hours. Distilled water (1 mL) was added to the dried precipitates and boiled for 1 hour. After centrifugation (22,300 × g, 5 min, 4℃), the supernatant was mixed with 6 mL of 12.5 mM sodium tetraborate dissolved in 95% sulfuric acid. After boiling for 5 min, the sample solution was vigorously mixed with 0.1 mL of 0.15% NaOH (w/v) for 5 min and kept at room temperature for 20 min. The absorbance at 540 nm was measured; a pectin standard (Fluka, catalog #76280) was used for quantification.

Cellulose; The homogenized samples (3 mL) were centrifuged and the precipitates were mixed with 5 mL of cellulase solution. The cellulase solution was prepared by dissolving 80 mg of cellulase (1.92 U mg^{-1}, Sigma, catalog # C1794) in 5 mL of 0.1 M sodium acetate buffer, pH 5. The mixtures were incubated for 20 h at 37℃ and centrifuged (29,000 × g, 5 min). The reducing sugar contents in supernatants were measured by the DNS method at 540 nm (Kim et al., 1986).

Crude protein; The quantity of crude protein was measured by the method of Bradford (Bradford, 1976). The CC leaves were ground with liquid nitrogen in a mortar and pestle. The ground sample (0.2 g) was mixed with 1 mL of Tris buffer (50 mM, pH 7.5). After mixing with a vortex mixer, the sample solution was kept on ice for 10 min. After centrifugation (700 × g, 5 min, 4℃), the supernatant was used for protein quantification. The Bradford reagent (Bio-Rad, catalog # 500-0006) was mixed with the sample and absorbance at 595 nm was measured. Bovine serum albumin was used as the standard.

Crude fat; The homogenized samples (3 mL) were serially mixed with 5 mL of acetone, 7 mL of hexane, and 5 mL of 20% (w/v) NaCl. The mixture was centrifuged (1,000 × g, 15 min). The hexane layer was dried at 70℃. The weight after drying was determined as crude fat.

Vitamin C; Vitamin C was measured by the 2,4-dinitrophenyl hydrazine (DNP) method (AOAC, 1995). The CC leaves (1 g) were homogenized with 10 mL of metaphosphoric acid (5%, v/v). Metaphosphoric acid was further added into the sample solution to a final volume of 25 mL. Then 2 mL of this sample solution was mixed with 1 mL of 2,6-dichlorophenol indophenol and 2 mL of thiourea solution (2% thiourea in metaphosphoric acid). One mL of DNP solution (2 g of 2,4-dinitrophenyl hydrazine in 100 mL of 9 N H_2SO_4) was added and the reaction mixture held at 50℃ for 70 min. Sulfuric acid (5 mL, 85%, v/v) was added to stop the reaction. For the blank, sulfuric acid was added before the DNP solution. The absorbance of samples and blanks were measured at 520 nm.

Vitamin E; The content of vitamin E was measured by the modified method of Aaran and Nikkari (Aaran et al., 1988). α-Tocopherol (20 $mg\cdot mL^{-1}$; Sigma catalog no T3634) in 100 mL of absolute ethanol was used for the standard. Leaf samples (2 g) were ground by mortar and pestle in liquid nitrogen, and mixed with 3 mL of absolute ethanol, 3.1 mL of 10% pyrogallol solution (w/v, in ethanol), and 0.1 mL of 90% KOH (w/v). For saponification, the mixture was incubated at 65℃ for 30 min. After cooling, the sample was mixed with 4 mL of distilled water and 3 mL of hexane. After centrifugation (1,400 × g, 3 min), the hexane layer was repeatedly washed with distilled water until the color of the water was not changed by phenolphthalein reagent. Anhydrous Na_2SO_4 was added to remove water remaining in the hexane solution. After evaporation of the hexane at 75℃, 2.5 mL of absolute ethanol was used to dissolve vitamin E. The sample solution (0.5 mL) was mixed with 0.1 mL of 2% $FeCl_3$ solution (w/v, in ethanol) and 0.1 mL of 0.5% 2,2'-dipyridyl solution (w/v, in ethanol), and 1.8 mL of absolute ethanol. The absorbance of the final solution was measured at 520 nm.

Glucosinolate; The homogenized sample (3 mL) was mixed with 4 mL of 0.1 M phosphate citrate buffer (pH 7.0). Butyl isothiocyanate was used as the internal standard at 50 nmol·mL^{-1}. The sample was mixed with 2mL of dichloromethane (DCM) and 0.3 U of thioglucosidase (Sigma, catalog # T4528). After incubation at 30℃ for 3 h with mild shaking, the samples were centrifuged (2,100 × g, 10 min), and the upper DCM layer was collected. To remove residual water, 0.5 g of sodium sulfate was added and centrifuged (2,100 × g, 10 min). The samples were filtered (0.45 µm PTFE membrane filter, Millipore, Billerica, MA). To identify isothiocyanates, GC-MS (gas chromatography-mass spectrometry) was used (HP 5890 GC/5972MSD, Palo Alto, CA), with a DB-5MS column (0.25 mm × 30 m, 0.25 µm, Agilent, Santa Clara, CA). Injector and detector temperatures were 250℃ and 300℃, respectively. Helium was used as the carrier gas at a flow rate of 1 mL·min^{-1}. The column oven temperature program was 35℃ for 1 min, 1℃/min up to 45℃, 3℃/min up to 180℃ and 20℃/min up to 300℃. For routine analysis, GC-FPD (Flame photometric detector detecting S compounds; Shimadzu GC-14B, Kyoto, Japan) and GC-NPD (nitrogen-phosphorous detector detecting N compounds; Agilent Technologies, 6890N, Santa Clara, USA) were used under the same conditions as for GC-MS.

Statistical analysis

SPSS 14.0 was used for statistical analysis. For average comparison between two groups, one-way ANOVA were performed. Pearson correlation coefficients were calculated for the correlations between dependent variables.

Results and Discussion

The pH of soil was determined by measuring the pH after addition of 10 volumes of distilled water. The pH of the soil in the greenhouse was 6.2, whereas that of the outdoor soil was 7.6 (Table 2). Components of the 13 cultivars were analyzed to measure the effect of soil pH. In this study, the leaves of the third outer layer of CC (the first layer of edible leaves) were used for comparison.

Nutritional components

Moisture contents of CC cultivated on the soil of pH 6.2 (CC-6.2) were higher than those of CC cultivated on the soil of pH7.6 (CC-7.6) (Table 3). The CC cultivars containing increased moisture content showed a soft texture (data not shown). Reducing sugars in CC-6.2 were 65% of those in CC-7.6 (Table 3). The reducing sugars, including glucose, are important for the quality of CC because they affect the taste. A high reducing sugar content could also cause fast fermentation (acetic and lactic acid production) in Kimchi

Table 3. Comparison of nutritional components in 13 Chinese cabbage cultivars cultivated on soils of different pHs.

Access^z number	pH 6.2	pH 7.6	pH 6.2	pH 7.6	pH 6.2	pH 7.6
	Moisture** (%)		Reducing Sugar** (mg·g^{-1} wet wt)		Crude Protein** (mg·g^{-1} wet wt)	
26139	88.1 ± 2.0	91.1 ± 0.2	23.2 ± 0.3	36.8 ± 2.0	2.5 ± 0.3	1.0 ± 0.0
26142	87.5 ± 0.4	91.1 ± 1.0	21.9 ± 0.1	31.1 ± 2.5	2.3 ± 0.3	0.8 ± 0.0
26145	84.2 ± 1.1	92.3 ± 0.9	21.8 ± 0.5	20.9 ± 1.5	2.2 ± 0.2	1.1 ± 0.0
26146	86.9 ± 0.3	92.6 ± 0.6	25.0 ± 0.1	24.9 ± 1.6	1.5 ± 0.2	1.4 ± 0.0
26148	88.7 ± 1.0	85.1 ± 2.3	16.1 ± 0.3	29.4 ± 0.6	2.2 ± 0.3	0.6 ± 0.0
26151	84.9 ± 0.8	89.8 ± 0.8	23.9 ± 0.3	29.8 ± 2.3	2.3 ± 0.3	1.1 ± 0.0
26152	87.6 ± 0.7	87.2 ± 0.4	18.5 ± 0.4	41.4 ± 0.9	1.5 ± 0.2	1.4 ± 0.1
26153	87.4 ± 1.0	91.4 ± 0.3	20.0 ± 0.1	35.5 ± 0.8	2.4 ± 0.1	1.2 ± 0.0
26156	86.9 ± 0.2	91.5 ± 0.6	22.2 ± 0.4	36.9 ± 2.6	3.0 ± 0.1	1.0 ± 0.1
26161	87.3 ± 1.0	92.3 ± 0.4	23.1 ± 0.3	39.4 ± 3.7	2.1 ± 0.4	1.3 ± 0.0
26164	89.1 ± 1.2	90.8 ± 0.3	20.0 ± 0.3	29.6 ± 1.8	2.5 ± 0.2	0.7 ± 0.0
26168	89.3 ± 0.8	92.8 ± 0.2	19.4 ± 0.3	32.5 ± 2.6	1.6 ± 0.1	0.9 ± 0.0
26169	88.6 ± 0.5	91.2 ± 0.3	14.1 ± 0.3	27.4 ± 0.4	2.4 ± 0.0	1.3 ± 0.0
Average^y	87.4 ± 1.7	90.7 ± 2.3	20.7 ± 3.1	32.0 ± 6.0	2.2 ± 0.5	1.1 ± 0.3
Ratio	0.96		0.65		2.07	

(Hong and Kim, 2006; Jung et al., 1985).

The crude protein contents of CC-6.2 were two-fold higher than those in CC-7.6 (Table 3). However, the crude fat content in CC-6.2 was about half that of CC-7.6 (Table 3). Interestingly, cultivar #26168 showed a reverse trend in fat content compared to other cultivars.

CC-6.2 contained significantly increased vitamins C and E compared to CC-7.6 (Table 3). Vitamins C and E are important antioxidants for human beings (Schneider, 2005): the recommended daily allowance for vitamin C is 60 mg/day and for vitamin E is 15 mg/day (Wardlaw, 1999). Thus, a person consuming more than 60 g of cultivars #26148 or #26152 would be provided with their daily requirement of vitamin C. Similarly, 60 g of cultivar #26152 would provide a daily requirement of vitamin E. Therefore, regarding the nutritional aspect of vitamin content, CC-6.2 was higher in

Table 3. Continued.

Access number[z]	pH 6.2	pH 7.6	pH 6.2	pH 7.6	pH 6.2	pH 7.6
	Crude Fat** (mg·g^{-1} wet wt)		Pectin** (mg·g^{-1} wet wt)		Cellulose** (mg·g^{-1} wet wt)	
26139	27.7 ± 3.8	52.3 ± 41.7	11.0 ± 0.6	2.5 ± 0.1	27.5 ± 0.9	81.1 ± 2.9
26142	24.0 ± 6.6	67.7 ± 2.3	7.2 ± 0.4	0.5 ± 0.0	31.4 ± 1.0	55.5 ± 3.1
26145	28.0 ± 4.0	60.0 ± 1.7	9.6 ± 0.3	0.8 ± 0.1	28.5 ± 0.2	55.5 ± 1.8
26146	23.3 ± 5.5	75.0 ± 3.0	13.7 ± 0.2	0.7 ± 0.0	33.0 ± 2.1	66.6 ± 3.0
26148	24.3 ± 1.5	60.3 ± 2.1	8.0 ± 0.2	1.1 ± 0.0	28.9 ± 1.0	71.5 ± 3.0
26151	6.3 ± 0.6	48.0 ± 1.0	9.3 ± 0.0	0.8 ± 0.1	37.4 ± 0.1	59.1 ± 3.0
26152	15.3 ± 2.9	49.7 ± 0.6	9.9 ± 0.2	0.8 ± 0.0	30.6 ± 0.5	80.0 ± 1.5
26153	19.0 ± 3.6	44.7 ± 1.5	7.9 ± 0.3	1.1 ± 0.0	27.3 ± 1.5	59.4 ± 2.6
26156	19.3 ± 2.9	69.0 ± 2.0	9.2 ± 0.0	1.1 ± 0.1	30.8 ± 1.7	62.0 ± 3.6
26161	48.3 ± 6.8	68.3 ± 4.5	7.9 ± 0.0	1.0 ± 0.1	34.7 ± 0.2	72.4 ± 3.8
26164	30.0 ± 8.5	52.3 ± 1.5	8.2 ± 0.1	1.6 ± 0.2	32.6 ± 0.2	55.0 ± 3.5
26168	35.0 ± 8.7	13.7 ± 1.5	6.2 ± 0.1	0.7 ± 0.1	45.7 ± 0.4	67.6 ± 2.6
26169	46.7 ± 7.5	56.7 ± 3.1	5.0 ± 0.1	0.6 ± 0.1	42.9 ± 0.2	50.6 ± 2.3
Average[y]	26.6 ± 12.3	55.2 ± 18.0	8.7 ± 2.1	1.0 ± 0.5	33.2 ± 5.7	64.3 ± 9.8
Ratio	0.5		8.6		0.5	

Access number[z]	pH 6.2	pH 7.6	pH 6.2	pH 7.6	pH 6.2	pH 7.6
	Vitamin C** (μg·g^{-1} wet wt)		Vitamin E** (μg·g^{-1} wet wt)		Ash* (mg·g^{-1} wet wt)	
26139	851.8 ± 62.1	220.5 ± 0.8	122.5 ± 13.0	17.6 ± 1.6	13.6 ± 0.6	11.7 ± 1.1
26142	664.4 ± 114.3	192.0 ± 5.6	116.3 ± 1.9	7.3 ± 0.9	13.5 ± 1.3	13.7 ± 1.5
26145	872.3 ± 52.4	11.8 ± 0.2	89.0 ± 19.0	7.0 ± 1.4	19.1 ± 0.8	6.7 ± 0.8
26146	866.6 ± 40.4	172.5 ± 2.3	58.8 ± 4.6	4.0 ± 0.6	15.6 ± 0.8	13.4 ± 1.6
26148	1074.3 ± 20.2	182.7 ± 0.5	92.3 ± 14.1	8.5 ± 0.7	19.7 ± 5.4	9.6 ± 2.1
26151	705.1 ± 47.5	301.1 ± 5.0	148.3 ± 7.7	16.1 ± 0.8	15.8 ± 3.8	8.5 ± 1.7
26152	1082.8 ± 31.8	300.8 ± 14.1	184.9 ± 0.6	15.0 ± 0.4	18.5 ± 3.6	15.6 ± 1.5
26153	591.7 ± 14.1	517.8 ± 9.5	122.2 ± 0.7	8.9 ± 0.4	13.9 ± 1.4	14.5 ± 4.4
26156	845.3 ± 30.3	93.2 ± 1.4	151.8 ± 6.8	8.0 ± 0.3	21.9 ± 2.6	4.9 ± 0.2
26161	626.8 ± 21.8	39.4 ± 3.0	92.6 ± 0.8	38.4 ± 0.6	13.8 ± 5.8	15.7 ± 1.8
26164	573.9 ± 4.0	257.0 ± 1.6	125.7 ± 5.7	31.0 ± 3.8	25.5 ± 2.8	5.5 ± 0.4
26168	446.6 ± 5.6	197.1 ± 7.5	121.7 ± 6.6	7.5 ± 1.2	25.3 ± 2.1	7.3 ± 1.0
26169	443.9 ± 27.3	308.6 ± 0.4	83.1 ± 10.6	24.5 ± 3.7	23.2 ± 2.9	31.0 ± 1.6
Averagey	733.0 ± 202.9	215.0 ± 128.1	116.1 ± 33.5	14.9 ± 10.4	18.4 ± 5.0	12.2 ± 6.8
Ratio	3.4		7.8		1.5	

[z] Access number in Korea Brassica Genome Resource Bank (Daejeon, Korea).

[y] Average: t-test of all average comparisons between the two conditions were all statistically significant ($p<0.01$).

**, *Average difference by paired t-test was significant at the level of $p<0.01$ or $p<0.05$.

quality than CC-7.6.

Texture-related dietary fibers

Examining dietary fiber, the contents of pectin and cellulose were analyzed. CC-6.2 in the greenhouse contained significantly increased pectin by 8.7-fold, compared to CC-7.6 (Table 3). Cellulose levels in CC-6.2 were about half that of CC-7.6 (Table 3). Both pectin and cellulose are important dietary fibers (Khokhar et al., 2005; Wardlaw, 1999) but the effect on texture is quite different. Pectin usually gives a good texture but cellulose gives a woody mouth-feel. Therefore, regarding the texture, CC-6.2 had better characteristics than CC-7.6. However, from the standpoint of total dietary fibers, CC-7.6 had a higher value than CC-6.2.

Ash and minerals

The ash contents of CC-6.2 were significantly higher (1.5-fold) than those of CC-7.6 (Table 3). Iron, zinc, copper, calcium, magnesium, selenium and iodine are frequently deficient in human diets (White et al., 2009). Therefore, the levels of several important minerals were determined in this study.

Though the difference in Ca content between two soils was not significant (Table 2), 11 out of 13 cultivars on the acidified soil contained more Ca (Table 4). Interestingly, cultivars #26156 and #26164, cultivated on the soil of pH 7.6, also contained relatively large amounts of Ca and Mg (Table 4). These cultivars are recommended for the nutritional sources of Ca and Mg.

The acidified soil released 3-4 fold increased contents of Fe, Na, and Zn in 0.1N HCl compared to in distilled water (Table 2). Contents of these salts in CC-6.2 were higher than those in CC-7.6 by 11.3-, 5.6-, and 1.8-fold, respectively (Table 4). In the soil of pH7.6, cultivar #26148 contained a relatively large amount of Ca and Mg, and an outstanding content of Zn (Table 4).

The K levels in CC were not significantly affected by the pH of soil. Interestingly, the contents of Mn in the soils were not significantly different but were significantly higher in CC-6.2 than in CC-7.6 (Table 4). The Mn levels in CC-6.2 varied widely compared to other minerals. The Mn-accumulating cultivars, such as #26139, might be useful for bioremediation of excess Mn in soil.

Vegetables are important sources of minerals needed for human nutrition (Bettger, 1993; Hardisson et al., 2001) but some heavy metals can be toxic (Savas et al., 1995). In this study, the Co, Cd, Pb and Cr contents were also measured, but they were not detected by our system except for very low levels of Cr (0.005-0.05 $mg \cdot g^{-1}$ wet weight; data not shown) and thus the cultivars used in this study are safe for human consumption. Boron deficiency can cause accumulation of pectins in cell wall (Yu et al., 2002). We measured the

Table 4. Comparison of mineral contents in 13 Chinese cabbage cultivars cultivated on soils of different pHs.

Access no	Ca**		Mg		Fe**		Zn		Na**		K		Mn*	
	pH 6.2	pH 7.6	pH 6.2	pH 7.6	pH 6.2	pH 7.6	pH 6.2	pH 7.6	pH 6.2	pH 7.6	pH 6.2	pH 7.6	pH 6.2	pH 7.6
	($mg \cdot g^{-1}$ wet wt)													
26139	3.1	0.2	0.4	0.1	0.2	0.0	0.0	0.0	0.3	0.1	3.5	2.7	0.1	0.0
26142	7.0	1.8	0.8	0.4	0.1	0.0	0.0	0.0	0.4	0.1	3.9	2.2	0.0	0.0
26145	5.2	0.4	0.8	0.2	0.2	0.0	0.0	0.0	0.7	0.1	5.1	3.8	0.0	0.0
26146	2.5	0.5	0.4	0.2	0.2	0.0	0.0	0.0	0.4	0.1	4.5	2.9	0.0	0.0
26148	3.0	3.3	0.3	0.6	0.2	0.0	0.0	0.0	0.6	0.1	3.3	4.3	0.0	0.0
26151	4.9	3.2	0.5	0.6	0.1	0.0	0.0	0.0	0.3	0.1	6.9	2.2	0.1	0.0
26152	6.5	0.4	0.6	0.2	0.2	0.0	0.0	0.0	0.3	0.1	5.5	3.2	0.1	0.0
26153	6.0	0.8	0.6	0.2	0.2	0.0	0.0	0.0	0.5	0.1	5.5	4.4	0.1	0.0
26156	5.5	6.0	0.5	1.6	0.2	0.0	0.0	0.0	0.5	0.1	2.5	2.7	0.0	0.0
26161	5.2	0.8	0.5	0.2	0.3	0.0	0.0	0.0	0.3	0.1	3.6	2.7	0.0	0.0
26164	5.0	4.6	0.5	1.1	0.2	0.1	0.0	0.0	0.4	0.1	3.0	5.4	0.0	0.0
26168	4.7	0.5	0.5	0.2	0.2	0.0	0.0	0.0	0.4	0.1	3.1	3.1	0.0	0.0
26169	4.6	0.2	0.5	0.2	0.1	0.0	0.0	0.0	0.5	0.1	3.4	3.4	0.0	0.0
Average	4.8	1.8	0.5	0.4	0.2	0.0	0.0	0.0	0.4	0.1	4.1	3.3	0.0	0.0
SD	1.4	1.9	0.1	0.4	0.0	0.0	0.0	0.0	0.1	0.0	1.3	0.9	0.0	0.0
Ratio	2.8		1.2		11.3		1.8		5.6		1.3		6.2	

**, *Average difference by paired t-test was significant at the level of $p<0.01$ or $p<0.05$.

contents of boron in several cultivars but there were no significant differences in boron contents between CC-7.6 and CC-6.2 (5 mg·g^{-1} wet weight CC; data not shown). Boron is an essential micronutrient for CC (Romheld et al., 1991) and this level was within the normal boron range of 30-150 mg·g^{-1} dry weight of CC (Moreno et al., 2003).

Glucosinolates

Glucosinolates are the source of isothiocyanates (ITCs), giving a pungent flavor in the Brassica family (Zang et al., 2009). Only two cultivars produced detectable amounts of sinigrin (detected as its ally ITC) but gluconasturtiin (detected as phenylethyl ITC) and gluconapin (detected as butenyl ITC) were found in most cultivars (Table 5). The level of allyl ITC was higher in CC-6.2 than in CC-7.6. Conversely, butenyl ITC and phenylethyl ITC were accumulated in CC-7.6 but not significantly in CC-6.2. Phenylethyl ITC is a very effective anticancer compound (Hayes et al., 2008; Wang et al., 2009). Considering the total amount of ITC and phenylethyl ITC in cultivar #26146, it can be recommended as an anticancer CC (Table 5). However, glucosinolate at extremely high contents might hinder acceptance by consumers due to its bitterness, and thus a careful consideration is recommended.

The anticancer activity of phenylethyl ITC is excellent, as it induces cytoprotective genes mediated by Nrf2 and AhR transcription factors, represses NF-κB and inhibits cytochrome P450 and histone deactylase (Hayes et al., 2008; Lampe et al., 2002). Glucosinolates are produced from amino acids including tryptophan, tyrosine, phenylalanine, isoleucine, leucine, valine, alanine and methionine (Grubb et al., 2006). Phenylethyl ITC can be produced from phenylalanine and thus the contents of the amino acids in CC need to be measured for future breeding. Cultivar #26146 is recommended as a vegetable source of anticancer compounds and as a breeding stock for new cultivars (Table 5).

Correlations among measured factors

There was a positive correlation between reducing sugar and cellulose levels in CC-7.6 (Table 6) and, similarly, a positive correlation between reducing sugar and pectin in CC-6.2. Cellulose and pectin are polymers of sugar moieties and thus the production of these compounds requires sugar. There was a positive correlation between vitamin C and a negative correlation between vitamin C and cellulose in CC-6.2. In strawberries, vitamin C is produced from D-galacturonic acid, a principal component of pectins (Agius et al., 2003). Therefore, if carbon flow to pectin was increased, more D-galacturonic acid would be available and this may have

Table 5. Glucosinolates in 13 Chinese cabbage cultivars from acidified and neutral soil.

Access no^z	Ally isothiocyanate		Butenyl isothiocyanate^y, **		Phenethyl isothiocyanate**	
	pH 6.2	pH 7.6	pH 6.2	pH 7.6	pH 6.2	pH 7.6
	(nmol·g^{-1} wet wt)					
26139	NDa	ND	ND	63.3	ND	34.7
26142	ND	ND	5.2	113.6	2.8	28.1
26145	1.4	0.5	0.1	178.0	ND	22.9
26146	ND	ND	0.4	229.0	0.3	90.3
26148	ND	ND	0.9	79.9	0.5	14.2
26151	ND	ND	3.0	178.6	2.6	25.9
26152	ND	ND	15.0	330.1	6.0	11.3
26153	ND	ND	0.5	159.3	0.6	0.7
26156	ND	ND	0.3	158.2	0.7	ND
26161	ND	ND	0.2	163.8	0.3	2.0
26164	3.4	ND	0.4	36.0	0.8	2.7
26168	ND	ND	0.2	118.1	0.5	0.2
26169	ND	ND	ND	82.0	ND	5.3
Average	0.4	0.0	2.0	145.4	1.2	18.3
SD	± 0.1	± 0.2	± 4.2	± 77.9	± 1.7	± 24.7

^{z}Access number in Korea Brassica Genome Resource Bank (Daejeon, Korea).

^{y}Butenyl isothiocyanate: tentatively identified as butenyl isothiocyanate by GC-MS.

**Average difference by paired t-test was significant at the level of p<0.01.

NDNot detected.

Table 6. Pearson correlation coefficients among components of Chinese cabbage.

	Moisture	Reducing Sugar (RS)	Crude Protein (CP)	Crude Fat (CF)	Pectin	Cellulose	Vitamin C (VC)	Vitamin E (VE)	Ash	Butenyl isothiocyanate (B-ITC)	Phenylethyl isothiocyanate (P-ITC)	Ca	Mg	Fe	Zn	Na	K	Mn
Moisture									**pH 6.2**									
RS	-0.6																	
CP	-0.1	0.0																
CF	0.4	-0.3	0.0															
Pectin	-0.4	0.7**	-0.3	-0.5														
Cellulose	0.3	-0.3	-0.3	0.4	-0.5													
VC	-0.3	0.1	-0.2	-0.5	0.6*	-0.7*												
VE	0.0	0.0	0.1	-0.6*	-0.1	-0.1	0.2											
Ash	0.4	-0.5	0.0	0.3	-0.4	0.5	-0.3	0.1										
B-ITC^{z}	0.0	-0.1	-0.4	-0.4	0.1	-0.1	0.5	0.7*	-0.1									
P-ITC	-0.1	0.0	-0.4	-0.5	0.1	-0.1	0.4	0.7**	-0.1	1.0**								
Ca	-0.2	-0.1	0.1	-0.1	-0.4	0.0	-0.2	0.6*	-0.1	0.5	0.6*							
Mg	-0.5	0.2	0.1	-0.2	-0.1	-0.2	-0.1	0.2	-0.2	0.3	0.3	0.8**						
Fe	0.1	0.2	0.2	0.1	0.2	-0.4	0.2	0.2	-0.1	0.0	0.0	0.2	-0.1					
Zn	-0.4	0.3	0.3	-0.5	0.1	0.1	0.0	0.5	-0.2	0.1	0.2	0.2	-0.1	0.0				
Na	-0.2	-0.4	0.2	0.1	-0.2	-0.2	0.1	-0.4	0.3	-0.4	-0.4	-0.1	0.3	-0.2	-0.5			
K	-0.7*	0.3	-0.3	-0.6*	0.3	-0.2	0.2	0.2	-0.5	0.4	0.5	0.2	0.3	-0.3	0.4	-0.1		
Mn	0.0	0.2	0.1	-0.4	0.2	-0.3	0.2	0.6*	-0.3	0.3	0.3	0.0	-0.1	0.0	0.5	-0.4	0.3	

	Moisture	Reducing Sugar (RS)	Crude Protein (CP)	Crude Fat (CF)	Pectin	Cellulose	Vitamin C (VC)	Vitamin E (VE)	Ash	Butenyl isothiocyanate (B-ITC)	Phenylethyl isothiocyanate (P-ITC)	Ca	Mg	Fe	Zn	Na	K	Mn
Moist.									**pH 7.6**									
RS	-0.2																	
CP	0.3	0.2																
CF	0.0	-0.2	0.1															
Pectin	-0.1	0.3	-0.3	0.0														
Cellulose	-0.4	0.6*	0.2	-0.1	0.5													
VC	-0.2	0.2	0.2	-0.4	0.0	-0.1												
VE	0.1	0.3	0.1	0.1	0.3	0.0	-0.1											
Ash	0.0	0.0	0.5	0.1	-0.3	-0.1	0.3	0.3										
B-ITC	-0.1	0.2	0.7**	0.1	-0.4	0.3	0.0	-0.3	0.0									
P-ITC	0.2	-0.4	0.2	0.4	-0.1	0.1	-0.2	-0.4	0.0	0.3								
Ca	-0.3	0.1	-0.6*	0.2	0.1	-0.3	-0.1	0.0	-0.6	-0.3	-0.3							
Mg	-0.1	0.1	-0.5	0.2	0.2	-0.3	-0.2	0.1	-0.5	-0.2	-0.3	1.0**						
Fe	-0.1	-0.1	-0.5	0.0	0.2	-0.4	0.2	0.3	-0.5	-0.4	-0.3	0.8**	0.7**					
Zn	-0.8**	-0.1	-0.5	0.1	0.1	0.2	0.1	-0.2	-0.1	-0.2	0.0	0.3	0.2	0.1				
Na	-0.2	0.2	-0.5	0.3	0.6*	0.3	-0.5	0.6*	-0.3	-0.4	0.0	0.3	0.2	0.3	0.3			
K	-0.2	-0.2	-0.3	-0.2	0.2	-0.2	0.3	0.2	-0.1	-0.3	-0.3	0.2	0.2	0.6	0.4	0.2		
Mn	-0.1	-0.1	-0.5	0.0	0.3	-0.4	0.1	0.3	-0.5	-0.4	-0.2	0.8**	0.8**	0.97**	0.1	0.4	0.4	

^{z}ITC: isothiocyanate.

*, **Significant at the level of $p<0.05$ or $p<0.01$.

caused the increased production of vitamin C. If carbon flow were directed to cellulose, there might be a lower production of pectins. This hypothesis was supported by the correlation coefficient between pectin and cellulose (-0.51) at p=0.076 (Table 6).

Ca and Mg levels were strongly correlated with each other on both soils (Table 6). These minerals are important components of bone (Wardlaw et al., 1999). They prevent absorption of lipids by saponification of fatty acids (Vaskonen, 2003), meaning healthful cardio-vascular system. Several cultivars, e.g., #26156 and #26164, were a good source of Ca, Fe, and Mg, which are deficient in many diets and important for pregnant women (White et al., 2009).

The levels of Ca, Fe, Na, and Mn were significantly ($p<0.05$) affected by the soil pH (Table 4). Therefore, measurement of these minerals must be performed with consideration to the soil pH. If the soil pH is <5.5, plant absorbs less phosphate, and if >6.6 plant contains less Fe, Mn, Zn, Cu and B (Personal communication; Choi, Jong-Myung, Chungnam National University). This factor explains the higher contents of Fe, Zn, Mn in CC-6.2 than in CC-7.6 (Table 4) and also the high correlations between these minerals in CC-6.2 (Table 6). Other significant correlations remain unknown at this time, and thus further study of the physiology of CC is required.

In this study, the number of cultivars investigated was limited and thus the effect of genetic variation was not measured. However, the variations we did measure between cultivars were significant. For example, the variation of crude fat in CC-6.2 was 6-47 $mg \cdot g^{-1}$ wet weight (Table 3), and that of phenylethyl isothiocyanate in CC-7.6 was 0-90 $nmol \cdot g^{-1}$ wet weight (Table 5). Seasonal effects on the composition of another Brassica rapa, pakchoi, caused about 1.5-fold differences in total carotenoids, ascorbic acid, calcium, and total glucosinolates (Hanson et al., 2009). Compared to the seasonal results, the effect of soil pH on the levels of these components in CC was extremely significant, as shown in Table 3-5. However, reports of studies on these topics have been unavailable to date. In this report, we showed that the effect of soil pH. Our group is preparing a systematic analyses for CC from China. Before we start a complete analyses, we tried to remove environmental effect on components of CC. Therefore, in this study, two lands were selected and the soil pH had been compared. To remove the effect of soil pH, 13 samples were selected and their components were compared. The pH of the soil in the green house was lower than outdoor soil. To make similar conditions, the sides of green house were completely open. We showed that the effect of soil pH on the content ratios of CC-6.2 to CC-7.6 were, on average, as follows: moisture, 0.96; reducing sugar, 0.65; crude protein, 2.07; crude fat, 0.48; pectin, 8.57; cellulose, 0.52; vitamin C, 3.41; vitamin E, 7.78; ash, 1.51 (Table 3); Ca, 2.76; Mg, 1.19; Fe, 3.59; Zn, 1.80; Na, 5.63; K, 1.25; Mn, 6.22 (Table 4); butenyl ITC, 0.014; and phenylethyl ITC, 0.063 (Table 5). The statistical comparison of averages showed that all chemical components and many minerals were significantly ($p<0.05$) affected by soil pH (Table 3 and 5). Several reports have indicated that the health-beneficial components of Brassica plants are fibers, vitamin C (Divisi et al., 2006), glucosinolates (Hayes et al., 2008), etc. The effect of soil pH on these components was dependent on genetic sources. For example, the vitamin C levels of cultivars #26148 and #26152 from the soil of pH 6.2 were similar (1,074 and 1,083 $\mu g \cdot g^{-1}$ wet wt, respectively) but those from the soil of pH 7.6 were quite different (173 and 301 $\mu g \cdot g^{-1}$ wet wt, respectively) (Table 3). Therefore, the content changes in vitamin C by soil pH were quite dependent on the cultivars. Considering functional components like glucosinolates, the variation between cultivars was more pronounced than for nutritional components like vitamin C (Table 3 and 5).

In conclusion, CC-6.2 in the greenhouse had a better texture than CC-7.6 because it contained increased pectin and lowered cellulose. CC-6.2 showed higher nutritional values because of high contents of protein, Ca, Fe, vitamin C and vitamin E. The high content of reducing sugar in CC-7.6 conversely indicated a good taste. CC-7.6 was also good as a nutraceutical vegetable because it contained large amounts of anticancer compounds such as glucosinolates.

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토양 pH가 배추(*Brassica rapa* ssp. *campestris*)의 영양성분과 기능성분에 미치는 영향

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초　록. 비닐하우스의 낮은 pH 토양(pH6.2)과 노지의 높은 pH 토양(pH7.6) 의 pH 차이가 배추 품질에 미치는 영향을 알아보기 위하여 13종 배추의 기능성 성분과 영양성분을 분석하였다. 낮은 pH 토양에서 재배된 배추는 높은 pH 토양에서 재배된 배추에 비하여 펙틴, 조단백질, 비타민 C, 비타민 E의 함량이 현저히 높았으며 회분과 무기질(Ca, Fe, Na, Mn) 또한 높았다. 그러나 환원당, 셀룰로오스, 조지방의 함량은 낮은 pH 토양의 배추가 높은 pH 토양의 것 보다 40-50% 낮았다. 글루코시놀레이트의 일종인 gluconasturtiin(18.33 vs. 1.16nmol·g^{-1} wet weight)과 gluconapin(145 vs. 2nmol·g^{-1} wet weight) 은 높은 pH 토양의 배추에서 낮은 pH 것보다 의미 있게 높았다. 본 연구 결과 낮은 pH 토양에서 재배된 배추는 펙틴의 함량이 높고 셀룰로오스의 함량이 낮아 조직감이 좋으며 단백질, Ca, Fe, vitamin C와 E의 함량이 높아 영양성분이 향상 되었으나 높은 pH 토양에서 재배된 배추는 환원당과 글루코시놀레이트의 함량이 많아 맛과 항암성은 높음을 알 수 있었다.

추가 주요어 : 식이섬유, 글루코시놀레이트, 무기질, 비타민