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Phenolic compound profiles and their seasonal variations in new red-phenotype head-forming Chinese cabbages

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ABSTRACT

In previous study, red Chinese cabbages which satisfy the morphological characteristics of traditional Chinese cabbages were developed by crossing Chinese cabbage and red cabbage, with subsequent backcrossing. In this study, we evaluated the phenolic compound profiles of two newly developed red-phenotype Chinese cabbages in comparison with three typical cultivars. Anthocyanidins (cyanidin), phenolic acids (caffeic acid, *p*-coumaric acid, ferulic acid, and sinapic acid), and flavonols (quercetin and kaempferol) were identified and quantified by HPLC, LC/MS and LC/MS/MS analyses. The new cultivars contained significantly increased levels of phenolic compounds, except for kaempferol. Their contents drastically varied by sowing season. The spring-sown red Chinese cabbages contained significantly higher levels of phenolic acids (11,530 and 12,437 μg/g dry wt.) and cyanidin (1830 μg/g dry wt.) but lower levels of flavonols than their fall-sown counterparts. Correlation analysis exhibited a significant positive correlation between cyanidin and phenolic acids but a negative correlation between phenolic acids and flavonols in red Chinese cabbages. Principal component analysis could differentiate red Chinese cabbages and typical cultivars with different sowing seasons based on their phenolic compound profiles. This study provides information regarding the selection of appropriate Chinese cabbage cultivars rich in phenolic compounds that may benefit human health.

1. Introduction

Chinese cabbage (*Brassica rapa* L. ssp. *pekinensis*) is widely grown in Asia and is one of the most important vegetables in Korea, Japan, and China, although its consumption has gradually increased in Western countries. This vegetable has a short growing period and high nutritional and functional values, which can offer high economic benefits. Chinese cabbage is sold mainly for fresh use in stir-fry meals and other dishes. In Korea, Chinese cabbage is the principal ingredient of kimchi, a traditional fermented food that is consumed every day. Because of its wholesome nutritional profile, kimchi was selected as one of the world's healthiest foods.

The regular consumption of vegetables has been found to be associated with improved health conditions (Gundgaard, Nielsen, Olsen, & Sorensen, 2003; Hung et al., 2004). The health-beneficial properties of vegetables can be largely ascribed to the presence of phytochemicals in the diet, and among the different phytochemicals present in *Brassica* vegetables, phenolic compounds seem to have the greatest potential of being beneficial to human health (Podsędek, 2007). Moreover, epidemiological evidence has supported the role that dietary phenolic compounds play in the prevention of cancers and cardiovascular diseases (Arts & Hollman, 2005; Pandey & Rizvi, 2009). At a molecular level, phenolic compounds scavenge free radicals (R·), resulting in the formation of stable phenolic radicals whose energy is not sufficient to promote oxidation in cellular components (Frankel, 2012; Rice-Evans, Miller, & Paganga, 1997). These antioxidants are known to help prevent oxidative stress-related disease and age-related disorders, such as cancer and cardiovascular and neurodegenerative diseases (Boots, Haenen, & Bast, 2008; Huxley & Neil, 2003; Nichenametla, Taruscio, Barney, & Exon, 2006; Owen et al., 2000).

Consumers' desires to either reduce the risk of or help manage health complications through an improved diet have stimulated

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research and development of agricultural and food products rich in bioactive factors. The members of the *Brassicaceae* family are rich food sources of antioxidants; therefore, to improve the quality of these vegetables, a recent breeding trend has been focusing on increasing the antioxidant potential of our daily food supply. To improve the nutritional and functional qualities of Chinese cabbages, pigments with antioxidant activity, such as anthocyanins and carotenoids, were genetically introduced by sophisticated breeding processes (Watanabe, Musumi, & Ayugase, 2011). The orange-colored Chinese cabbage has a characteristic deep orange-colored inner head and contains large amounts of carotenoids and phenolic compounds, which provides the desired antioxidative properties. In 2013, we reported a red-phenotype Chinese cabbage cultivar in which the red color was introduced from red bok choy. Metabolite profiling showed that the phenolic compounds in the red Chinese cabbage (RCC) were significantly higher than those in the regular green Chinese cabbages (GCC). However, the morphology of the cultivar did not match the traditional Chinese cabbage that is mostly consumed as the main ingredient for kimchi (Jiang et al., 2013).

Recently, we developed red-phenotype Chinese cabbage (RCC) cultivars by crossing Chinese cabbage and red cabbage. In this study, we compared phenolic compounds composition and content of the newly developed RCC with a conventional GCC. By comparing their contents in spring- and fall-sown Chinese cabbage, we also investigated how environmental conditions might affect phenolic compounds. Chinese cabbage cultivars and sowing seasons were differentiated and classified based on their phenolic compounds profile via statistical analyses.

2. Materials and methods

2.1. Chinese cabbages and culture conditions

Two Chinese cabbage cultivars developed and registered by the Kwonnong Seed Company (Cheongju, Korea) were used as red Chinese cabbages (RCC). RCC1 (trade name Kwonnongbbalgang No. 2) and RCC2 were induced by interspecific-crossing with green Chinese cabbage (*Brassica rapa* L. ssp. *pekinensis*) and red cabbage (*Brassica oleracea* L. var. *capitata* f. *rubra*), where allotetraploids were formed by using a colchicine treatment, and a recurrent backcrossing was employed for red aneuploid individuals of Chinese cabbage. The RCC showed a red phenotype with introduced dominant genes corresponding with red cabbage (data not shown; a detailed gene analysis will be published as an independent study). For the cultivation test and chemical analyses, GCC used as controls included GCC1 (trade name Bulam No. 3) and GCC2 (trade name Hwangsim) for the fall sowing and GCC3 (trade name CR-power Chunkwang) for the spring sowing, all of which are popular cultivars for kimchi. The Chinese cabbages were primarily planted in two sowing seasons (fall of 2013 and spring of 2014). The sowing day in the fall was the 2nd of August, and the plants were sampled on the 2nd of November. The spring samples were sown on the 15th of March and harvested on the 29th of May. The weather conditions, including average rainfall, temperature, and sunshine amount, are summarized in Supplemental Fig. 1. After the cabbages were harvested, they were freeze-dried, powdered with a grinder, and stored at −70 °C until analysis. Three cabbages were harvested from each cultivar, and all analyses were performed in duplicate at a minimum.

2.2. Chemicals

Cyanidin chloride, delphinidin chloride, malvidin chloride and pelargonidin chloride were purchased from Extrasynthese (Lyon, France) as anthocyanidin standards. Caffeic acid, *p*-coumaric acid, ferulic acid, sinapic acid, quercetin and kaempferol were obtained from Sigma-Aldrich (St. Louis, MO, USA). Extraction solvents were of analytical grade or higher. Acetonitrile and methanol were from J.T. Baker (Phillipsburg, NJ, USA) and used for HPLC mobile phase.

2.3. Sample preparation and extraction for anthocyanidin, phenolic acid, and flavonol analysis

Freeze-dried Chinese cabbage samples were ground in a blender and 0.1 g samples were used for extraction. Anthocyanins were extracted and hydrolyzed to anthocyanidins using the method described by Zhang, Kou, Fugal, and McLaughlin (2004). Briefly, the sample was homogenized in 4 mL of 50% methanol (v/v, containing 2 M HCl) for 20 min. Then mixture was incubated at 100 °C for 1 h in a water bath, and the hydrolyzed sample was immediately cooled in ice. For phenolic acid extraction, the ground sample was mixed with 6 mL of 53% methanol (v/v, containing 2.6 M NaOH) and incubated at 25 °C for 20 h in a shaking incubator. The pH of the sample solution was adjusted to pH 2 with HCl (Park et al., 2014). Flavonols were prepared according to the procedure published by Rochfort, Imsic, Jones, Trenerry, and Tomkins (2006). 0.1 g of sample was mixed with 4 mL of 62.5% aqueous methanol (v/v, containing 0.2% *tert*-butylhydroquinone) and homogenized for 20 min. Then 1 mL of 8 M HCl was added and mixture was incubated at 90 °C for 3.5 h in a water bath, and then cooled in ice. All the samples were filtered with 0.2 μm polypropylene filter (Advantec, Japan) before analysis.

2.4. HPLC-UV analysis

The identities of anthocyanidins, phenolic acids, and flavonols in the samples were confirmed by comparing their LC retention times, UV spectra, mass spectra and tandem mass spectra to those of authentic standards. Quantification was performed by high performance liquid chromatography-ultraviolet (HPLC-UV) detection. The instrument used in this study was UHPLC system (Ultimate 3000, Dionex, Idstein, Germany) with Ultimate 3000 RS diode array detector (Dionex, Idstein, Germany). Anthocyanidins, phenolic acids, and flavonols were analyzed separately as described below.

Anthocyanidin: Anthocyanidins were analyzed on a C18 column (Triart C18, particle size S-1.9 μm, 100x2 mm i.d.; YMC, Komatsu, Japan). Chromatographic separation was performed by a binary gradient consisting of solvent A (0.5% trifluoroacetic acid in water) and solvent B (acetonitrile). The solvent B gradient was 0% at 0 min, 14% at 2 min, 17% at 4 min, 28% at 7min, 36% at 9min, and 60% at 11min. The flow rate was set to 0.6 mL min^{-1} with a column temperature of 50 °C. Quantification was performed at 520 nm.

Phenolic acid: The instrument and column used for anthocyanidin analysis was employed for phenolic acid. The mobile phase was composed of solvent A (0.1% formic acid in water) and solvent B (methanol). The solvent B gradient was 20% at 0 min, 40% at 10 min, and 97% at 11 min. The flow rate was 0.45 mL min^{-1} with a column temperature of 35 °C. Spectral data were recorded at 330 nm.

Flavonol analysis: Flavonols were separated on a C18 column (Triart C18, particle size 5 μm, 250x4.6 mm i.d.; YMC, Komatsu, Japan), and the flow rate was set to 0.6 mL min^{-1}. Column temperature was maintained at 30 °C. The mobile phase was composed of solvent A (0.1% formic acid in water) and solvent B (methanol). The solvent B gradient was 50% at 0 min, 85% at 20min, and 95% at 30 min. Data were recorded at 360 nm.

2.5. HPLC/MS and HPLC/MS/MS analyses

Isolated phenolic compounds were further identified with LC/mass spectrometry (MS) and LC/tandem mass spectrometry (MS/MS). The LC/MS/MS system consisted of a Waters Xevo TQS triple quadrupole mass spectrometer connected to an Acquity UPLC system (Manchester, UK) through electrospray ionization (ESI) interface. The ESI was performed in both positive and negative ion modes for anthocyanidins and flavonols, and in negative ion mode for phenolic acids. The ESI parameters were set as follows: capillary voltage, 4500 V; cone voltage, 40 V; desolvation temperature, 600 °C; and desolvation gas flow, 650 L/