Ripening-dependent Changes in Phytonutrients and Antioxidant Activity of Red Pepper (*Capsicum annuum* L.) Fruits Cultivated under Open-field Conditions

Shiva Ram Bhandari¹ and Bo-Deul Jung

Department of Medical Biotechnology, Soonchunhyang University, Asan, 336-745, South Korea

Hum-Young Baek²

Korea Inspharm Inc., Dongmyon Daepori 1065 Hwasun, 519-882 South Korea

Young-Sang Lee²

Department of Medical Biotechnology, Soonchunhyang University, Asan, 336-745, South Korea

Additional index words. *Capsicum annuum*, vitamin, capsaicinoids, DPPH, maturity

Abstract. To understand ripening-dependent changes in phytonutrients, five commercial cultivars of red peppers (*Capsicum annuum* L.) grown in an open field in Taean, South Korea, were selected and their fruits were harvested at green mature (GM), intermediate breaker (BR), and red ripe (RR) stages and their phytonutrient contents and antioxidant activities were compared. Three major patterns in relation to ripening progress were observed. First, continuous increases were observed in vitamin C, total phenol, vitamin E (especially α -tocopherol), total free sugar, β -carotene, linolenic acid content, and antioxidant activity. Second, decreasing patterns were observed in phytosterols (campesterol, stigmasterol, and β -sitosterol) and linoleic acid. Third, total flavonoid and squalene contents were relatively higher at the BR stage compared with the GM and RR stages. These results indicate that each phytonutrient has a unique pattern of accumulation and degradation during the fruit-maturing process. Unlike the mentioned phytonutrients, which showed similar patterns in all tested cultivars, capsaicinoids exhibited quite different patterns of ripening-dependent changes among the cultivars. Throughout the ripening processes, positive correlations with antioxidant activity were observed in vitamin E ($r = 0.814^{**}$), β -carotene ($r = 0.772^{**}$), vitamin C ($r = 0.610^{**}$), and total phenol ($r = 0.595^{**}$) contents, whereas capsaicinoids, total flavonoid, and phytosterols exhibited no or slightly negative correlations. In conclusion, the ripening of red pepper fruits is accompanied by continuous increments in various phytonutrients and subsequent antioxidant activity.

Red pepper (*Capsicum annuum* L.), which is used as a pungent spice in foods and food products for flavoring and coloring, is one of the most widely consumed vegetables in the world (Garces-Claver et al., 2007). In South Korea, dried red pepper consumption per capita is ≈ 4.0 kg and red pepper is the most important horticultural crop in its cultivation area (50,454 ha), annual production (302,015 tons/year), and cultivated by over 55% of farmers in Korea [Korean Statistical Information Service (KOSIS), 2012]. The pepper

is a key ingredient in many kinds of important Korean processed food products such as *Kochujang* and *Kimchi* because of its unique pungency and color (Kim et al., 2002). Pepper contains a number of health-beneficial phytochemicals (Bhandari et al., 2012; Deepa et al., 2007; Topuz and Ozdemir, 2007). For example, capsaicinoid, a group of alkaloid compounds that is responsible for the pungency of pepper, possesses strong physiological and pharmacological activities (Mori et al., 2006). A number of phytochemicals that are present in peppers such as phenolics, vitamin C, vitamin E, and carotenoids may contribute to antioxidant activity and consequently show various pharmacological and nutritional activities (Balasundram et al., 2006; Burton and Traber, 1990; Guil-Guerrero et al., 2006; Khor and Chieng, 1997; Manach et al., 2005; Marangoni and Poli, 2010; Rietjens et al., 2002; van Rensburg et al., 2000; Zhuang et al., 2012). The amounts of these phytonutrient compounds, however, are readily affected by various genetic and environmental

factors such as the variety, cultivation practices, and even cultivation year (Bae et al., 2012; Deepa et al., 2007; Guerra et al., 2011; Howard et al., 2000; Marin et al., 2009; Menichini et al., 2009; Topuz and Ozdemir, 2007; Wahyuni et al., 2011). The ripening of pepper fruit is also an important factor in determining phytonutrient contents because during fruit ripening, pepper fruits undergo significant physiological, biochemical, and structural changes that in turn result in changes in color, flavor, firmness, and market quality (Cisneros-Pineda et al., 2007; Conforti et al., 2007; Deepa et al., 2007; Howard et al., 2000; Weryszko-Chmielewska and Michalajc, 2011). Therefore, it is important to understand how changes in various health-beneficial phytochemicals are influenced by the ripening process. Such ripening-dependent phytonutrient changes are especially important for the Korean diet because peppers harvested at green stages are popularly consumed as a fresh vegetable in Korea and its production area (26,398 ha) and annual market size ($\approx \$270$ million U.S.) reaches $\approx 10\%$ and 25% of the dried red pepper powder industry in Korea, respectively (KOSIS, 2012). Most previous phytonutrient research reports have focused on changes in capsaicin, water-soluble vitamin C, and carotenoids that are directly related to peppers' commercial quality such as pungency and color. In contrast, information about changes in lipid-soluble phytonutrients such as vitamin E, squalene, phytosterols, and fatty acids, which also carry important health-beneficial properties such as antioxidant, anticancer, tumor proliferative, and cholesterol-lowering effects (Burton and Traber, 1990; Hargrove et al., 2001; Khor and Chieng, 1997; Marangoni and Poli, 2010) in pepper fruits, is limited. This research focused on tracing ripening-dependent changes in various phytonutrients, including capsaicinoids, vitamin C, free sugars, total phenols, total flavonoids, vitamin E, squalene, campesterol, β -sitosterol, stigmasterol, and fatty acid composition, in pepper fruits and their relationships with antioxidant activity in five pepper cultivars most popularly cultivated in open fields of Taean county of South Korea.

Materials and Methods

Plant materials and sampling. In preliminary experiments, considerable variation in phytonutrients was observed among individual plants; i.e., pepper fruits of the same ripening stage that were collected from different plants exhibited significant variation (data not shown). To minimize individual plant-based variation in the evaluation of ripening-dependent variation, 10 to 15 individual pepper plants from five pepper cultivars: 21-Segi, Baerotta, Muhanjilju, PR-Mujeokplus, and Superbigarim, which were commercially cultivated in an open field in Taean, South Korea, were selected and pepper fruits were harvested from the plants at three different ripening stages: GM, BR, and RR. The sampled fruits were briefly cleaned

Received for publication 15 Apr. 2013. Accepted for publication 4 Aug. 2013.

This research is supported by a Soonchunhyang University Research Grant.

¹Present address: Department of Horticultural Crop Research, National Institute of Horticultural and Herbal Science, Rural Development Administration, Suwon, 440-706, South Korea.

²To whom reprint requests should be addressed; e-mail baekhy0323@hanmail.net; mariolee@sch.ac.kr.

with a paper towel and pooled together according to their ripening stage (≈ 1.5 kg for each stage) after their pedicel was removed. Some phytochemicals such as vitamin C, free sugars, total phenol, and total flavonoids and water content were measured using fresh samples. Vitamin E, squalene, phytosterols, fatty acids, β -carotene, and antioxidant activity were analyzed after the samples were dried in an oven at 55°C for 72 h and ground into a fine powder. The powdered samples were kept under airtight conditions at 4°C until the analysis was performed. In both the fresh weight as well as dry weight basis analyses, the whole pepper pod including all pericarp, placenta, and seeds was used for homogenization. All of the experiments were conducted in triplicate by collecting three samples for independent analyses from the pooled fresh or powdered samples.

Vitamin C analysis. Fresh peppers pods (5 g) were homogenized and extracted in 50 mL of 5% metaphosphoric acid solution. The extract was centrifuged, filtered through a $0.45\text{-}\mu\text{m}$ syringe filter (nylon), and analyzed using a high-performance liquid chromatography (HPLC) system (S1211; Sykam, Germany) with a Zorbax SB-Aq column (4.6×250 mm; $5\text{ }\mu\text{m}$; Agilent). As a mobile phase, 0.05 M metaphosphoric acid with acetonitrile (99/1, v/v) was used at a flow rate of $1.0\text{ mL}\cdot\text{min}^{-1}$. Measurements were taken using an ultraviolet detector (S3200; Sykam) at 254 nm . Data were recorded and integrated using MultichroTM (Yullin Technology, South Korea) chromatography software.

Total phenol analysis. Total phenolic content was estimated using the Folin-Ciocalteu colorimetric method based on the procedure of Singleton and Rossi (1965) using ferulic acid as a standard phenolic compound. Ten grams of fresh pepper were ground into a fine paste and extracted in 80% methanol for 12 h on an orbital shaker. Then, the extract was filtered through a Whatman No. 42 filter paper, and 1 mL of supernatant was mixed with 3 mL distilled water in a 15-mL Falcon tube. After adding 1 mL Folin reagent, the solution was incubated in a water bath at 27°C for 5 min. Then, 1 mL of saturated sodium carbonate was added. After 1 h, the absorbance of the extract was measured using a ultraviolet/VIS spectrophotometer (Biochrom-Libra S22, U.K.) at 640 nm . Ferulic acid standards in different concentrations were used for the calibration, and total phenol content was expressed as milligrams of ferulic acid equivalent (FAE) per 100 g of fresh pepper.

Total flavonoid analysis. Total flavonoid content was estimated using the colorimetric method as described by Marinova et al. (2005). The pepper extract that was obtained for the total phenol analysis was also used for the total flavonoid analysis. One milliliter of the extract was kept in a 15-mL Falcon tube containing 4 mL of distilled water. Then, 0.3 mL of 5% sodium nitrite was added; after 5 min, 0.3 mL of 10% AlCl_3 was added to the solution. At the sixth minute, 2 mL of 1 M NaOH was added and distilled water was added to obtain a final volume of 10 mL . The solution was mixed thoroughly and the absorbance was measured at 510 nm using a ultraviolet/

VIS spectrophotometer (Biochrom-Libra S22). Eighty percent methanol was used as a blank. Catechin hydrate in different concentrations was used as a standard, and total flavonoid was expressed as milligrams of catechin hydrate equivalent (CE) per 100 g of fresh weight.

β -carotene analysis. The β -carotene contents of pepper samples were determined following the method of Nagata and Yamashita (1992) with slight modifications. Fresh pepper fruits (1.0 g) were combined with liquid nitrogen, ground using a mortar and pestle, and transferred into 50-mL Falcon tubes. Then, 25 mL of acetone-hexane (4:6) solvent was added and the tube contents were mixed. The upper clear layer was taken and absorbance values at 663 , 645 , 505 , and 453 nm were measured using a ultraviolet/VIS spectrophotometer (Biochrom-Libra S22) with acetone-hexane (4:6) as a blank. The β -carotene content in a sample was calculated using the following formula:

$$\begin{aligned} \beta\text{-carotene (mg } 100\text{ mL}^{-1} \text{ of extract)} \\ = 0.216 * A_{663} - 1.22 * A_{645} - 0.304 * A_{505} \\ + 0.452 * A_{453} \end{aligned}$$

Free sugars analysis. Five grams of fresh ground pepper were extracted with distilled water by shaking for 20 min in a water bath at 80°C . The solution was centrifuged, filtered through a syringe filter ($0.45\text{ }\mu\text{m}$, nylon), and analyzed using the HPLC system (S1211; Sykam) with a Kromasil 100-10-NH2 column (250×4.6 mm) and a refractive index detector (S3500; Sykam) with acetonitrile/distilled water (75/25, v/v) as a mobile phase at a flow rate of $1.2\text{ mL}\cdot\text{min}^{-1}$.

Vitamin E, squalene, and phytosterols analysis. Samples for the vitamin E, squalene, and phytosterols analyses were prepared using the procedures described by Bhandari et al. (2012) with some modifications. Briefly, 2 g of powdered dried pepper was mixed with 15 mL ethanol and ascorbic acid as an antioxidant and shaken in a water bath at 80°C for 18 min. Next, $300\text{ }\mu\text{L}$ of 44% KOH was added and the mixture was shaken for 18 min at 80°C for saponification. The tubes were then immediately cooled in an ice bucket, 10 mL *n*-hexane and 10 mL of distilled water were added, the contents were mixed, the mixture was centrifuged for 10 min at 1000 rpm , and the upper hexane layer was collected. This process was repeated three times and the collected hexane layers were pooled and washed with 10 mL distilled water three times. The samples were then passed through anhydrous Na_2SO_4 to remove any water, concentrated in a rotary evaporator, dissolved in 1 mL isooctane, and injected into a gas chromatograph (GC; CP-3800; Varian) equipped with a flame ionization detector (FID) and a capillary column (CP-SIL 8CB, $30\text{ m} \times 0.25\text{ mm}$, $0.4\text{-}\mu\text{m}$ film thickness). The injector and detector temperature was set at 290°C and the injection volume was $1\text{ }\mu\text{L}$ with a split ratio of 1:20 under constant column flow ($1.0\text{ mL}\cdot\text{min}^{-1}$) with helium as a carrier gas. The oven temperature was initially set at 220°C for 2 min; it was then increased to 290°C at a rate of

$5^\circ\text{C}\cdot\text{min}^{-1}$, held constant for 14 min, and then increased to 310°C at a rate of $10^\circ\text{C}\cdot\text{min}^{-1}$.

Fatty acid composition analysis. Powdered pepper samples (0.2 g) were mixed with $680\text{ }\mu\text{L}$ of methylation mixture solution (MeOH:benzene:2,2-dimethoxypropane: $\text{H}_2\text{SO}_4 = 39:20:5:2$) and $400\text{ }\mu\text{L}$ of heptane. After vigorous mixing and heating for 2 h at 80°C in a water bath and cooling to room temperature, the heptane layer was collected and injected into a GC (GC-2010 plus; Shimadzu, Japan) equipped with a FID and a capillary column: CP-Sil 88 CB fatty acid methyl ester ($25\text{ m} \times 0.25\text{ mm}$, $0.25\text{ }\mu\text{m}$). Both the injector and detector temperatures were set at 210°C , and the injection volume was $1\text{ }\mu\text{L}$ with a 1:50 split ratio. A constant column flow ($1\text{ mL}\cdot\text{min}^{-1}$) of helium carrier gas was applied and the oven temperature was initially maintained at 100°C for 5 min, increased to 160°C at a rate of $5^\circ\text{C}\cdot\text{min}^{-1}$, maintained for 5 min, and increased to 180°C at a rate of $4^\circ\text{C}\cdot\text{min}^{-1}$.

Capsaicinoid analysis. Capsaicin and dihydrocapsaicin contents were analyzed using the methods described by Bhandari et al. (2012). Briefly, powdered pepper samples (2.0 g) were extracted with ethyl alcohol by shaking for 5 h at room temperature. After centrifugation, the supernatant was passed through a $0.45\text{-}\mu\text{m}$ nylon syringe filter and injected into an HPLC system (S1211; Sykam) equipped with an Eternity-5-Phenyl Hexyl column (150×4.6 mm; Alltech Co.) and acetonitrile/acetic acid/distilled water (40/1/59, v/v/v) as a mobile phase at a flow rate of $1.7\text{ mL}\cdot\text{min}^{-1}$. Measurements were taken using an ultraviolet/visible detector (ultraviolet-VIS 200; Linear) at 280 nm . The data were recorded and integrated using Peak Simple chromatography software (Version 3.56; SRI Instrument).

Determination of antioxidant activity. The antioxidant activities of pepper extracts were determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method of Koleva et al. (2002) with modifications. Powdered pepper samples (1.0 g) were extracted in 20 mL methanol for 12 h by shaking at room temperature and filtered through Whatman No. 42 filter papers. The aliquot was used to determine the samples' radical scavenging activity. First, $100\text{ }\mu\text{L}$ of DPPH solution was added to $100\text{ }\mu\text{L}$ of different concentrations (2.5 , 5.0 , 10.0 , 20.0 , and $30.0\text{ mg}\cdot\text{mL}^{-1}$) of extract in a 96-well plate. After 30 min, the absorbance levels of the resulting solutions were measured using a micro-plate reader (Infinite[®] M1000 PRO; TECAN, Austria) at 517 nm against methanol without DPPH as a blank. Similarly, the absorbance levels of samples were also measured after $100\text{-}\mu\text{L}$ samples were mixed with $100\text{ }\mu\text{L}$ of methanol. The free-radical scavenging activity (%) was calculated using the following equation:

$$\begin{aligned} \% \text{ of DPPH radical - scavenging activity} \\ = (B-A) \times 100/B \end{aligned}$$

where A is the absorbance of [(sample + DPPH) - (sample + methanol)] and B is

the absorbance of [(methanol + DPPH) – (methanol)].

IC₅₀ values, which represent the concentration required to obtain a 50% antioxidant capacity, were calculated and used to compare antioxidant activities among sample extracts.

Authentic standards and chemicals. Authentic standards for vitamin C, squalene, campesterol, stigmasterol, β -sitosterol, glucose, sucrose, fructose, ferulic acid, and catechin hydrate were purchased from Sigma-Aldrich. Capsaicin and dihydrocapsaicin were obtained from Fluka. Standards for fatty acid methyl ester were acquired from Supelco, and vitamin E (α -, β -, γ - and δ -tocopherols) standards were purchased from Merck (Germany). Chemicals such as acetonitrile, acetic acid, *n*-hexane, iso-octane, and methanol in HPLC grade were purchased from J.T Baker. Nitric acid and 2,2-dimethoxypropane were obtained from Sigma-Aldrich. Acetone, benzene, *n*-heptane, sulfuric acid, ethanol, sodium sulfate anhydrous, and sodium hydroxide were purchased from Daejung Chemicals (Republic of Korea). Chloroform and metaphosphoric acids were acquired from Junsei (Japan). Potassium hydroxide was purchased from Samchun (Republic of Korea), and ethyl ether (HPLC grade) was obtained from Fisher Scientific (Republic of Korea).

Statistical analyses. For each sample, at least three independent replicate measurements were taken. Data were expressed on either a fresh weight or dry weight basis; vitamin C, free sugars, total phenols, and total flavonoids were calculated on a fresh weight basis, whereas other phytonutrients were determined on a dry weight basis. General linear models for analysis of variance followed by Duncan's multiple range test at $P < 0.05$ (SPSS statistics, Version 18; SPSS, Chicago, IL) were used to evaluate significant difference in phytonutrients among ripening stages.

Results and Discussion

Vitamin C content. Vitamin C, a water-soluble natural antioxidant, is present in peppers in considerable amounts when compared with other vegetables (Lee and Kader, 2000). We observed higher vitamin C content in pepper cultivars ranging from 13.3 to 22.3 mg·kg⁻¹ at the green stage to 510.7 to 996.7 at the red stage than in red peppers from Turkey (Topuz and Ozdemir, 2007), sweet peppers (Deepa et al., 2007), and sweet bell peppers (Ghasemnezhad et al., 2011). Our results showed that vitamin C content was dependent on ripening with vitamin C increasing as ripening progressed (Fig. 1A). For example, 'Baerotta', a cultivar that had a relatively low vitamin C content compared with the other cultivars, showed continuous increment from the GM (13.3 mg·kg⁻¹) to the BR (360.1 mg·kg⁻¹) and RR stages (511.0 mg·kg⁻¹) exhibiting 38.4-fold increase. Similar ripening-dependent increases in vitamin C were found in all five tested cultivars. Even 'Muhanjilju', which had relatively higher vitamin C levels than the other cultivars, had low vitamin C contents in the GM stage (258.5 mg·kg⁻¹),

Fig. 1. Changes in (A) vitamin C, (B) total phenol, (C) total flavonoid, and (D) β -carotene contents in pepper fruits as affected by ripening stages. Vertical bars represent mean $\pm$ SD of three replicates, and different letters within the same cultivar indicate statistically significant difference at $P \leq 0.05$ by Duncan's multiple range test.

intermediate levels in BR (793.1 mg·kg⁻¹), and high levels in RR (996.7 mg·kg⁻¹). The ripening of pepper fruit has been thought to affect vitamin C content in sweet peppers (Deepa et al., 2007) and our results are consistent with previous reports by Howard et al. (2000) who described higher levels of vitamin C in various species of pepper fruits. However, inconsistent findings can also be found with some studies reporting that vitamin C increased or remained constant as fruit matured (Osuna-Garcia et al., 1998) and decreased with further ripening in red peppers (Deepa et al., 2007; Gnayfeed et al., 2001; Navarro et al., 2006) depending on the pepper species. These inconsistencies may be the result of differences in experimental conditions such as the cultivars used and sampling protocols. For example, in our experiment, we took samples from a relatively small number of pepper plants to reduce the plant-based variations, whereas other studies used pepper fruits from large numbers of plants as well as different species and cultivars, which would have led to the inclusion of more individual plant-based variation.

Total phenol content. Phenolic compounds, which are important secondary metabolites, possess various biological activities (Manach et al., 2005), the most important of

which is antioxidant activity that is associated with reduced cancer risk (Czeczot, 2000). Total phenol content in pepper fruits increased as ripening progressed; i.e., it was lowest in the GM stage, gradually increased in BR, and was highest in RR (Fig. 1B) in which it ranged from 276.9 to 938.4 mg FAE/kg in the GM stage to 938.4 to 1401.2 mg FAE/kg in the RR stage, which was higher than reported by Navarro et al. (2006) in red pepper and lower than reported by Howard et al. (2000) in various *Capsicum annuum* cultivars. All of the cultivars exhibited the same trend in total phenol increase, and among the five tested cultivars, 'PR-Mujeokplus' had the highest ripening-dependent increment (339% with 938.4 mg FAE/kg in the RR stage compared with 276.9 mg FAE/kg in the GM stage). These results are in agreement with those of Howard et al. (2000) and Lee et al. (1995) in various pepper species, although Conforti et al. (2007) and Ghasemnezhad et al. (2011) observed decreases in phenol content during ripening in bell pepper fruits. All these results suggest that total phenol content in red peppers is dependent not only on pepper genotype, but also by their ripening stages. However, further quantitative studies of individual phenolic compounds appear to be needed.

Total flavonoid content. Flavonoids are well-known natural antioxidants that serve as free radical scavengers to protect the human body from oxidative damage (Halliwell, 1996; Middleton et al., 2000; Miliauskas et al., 2004). Total flavonoid content also showed ripening-dependent changes. Unlike vitamin C and total phenol contents, however, total flavonoid content was higher in the BR stage than in the GM and RR stages (Fig. 1C) suggesting that phytonutrients have their own unique pattern of accumulation and degradation during the fruit-maturing process. Ripening-dependent changes in total flavonoid were not as prominent as the patterns in vitamin C and total phenols in that only three cultivars, 21-Segi, Baerotta, and Superbigarim, exhibited a statistically significant increase during the BR stage. These results are somewhat similar to those of Menichini et al. (2009), who observed a decrease in total flavonoid content in the RR stage of *Capsicum chinense* and the value reported in this study; 63.5 to 77.8 mg CE/kg in the GM stage to 68.9 to 96.9 mg CE/kg in the BR stage was higher than previously reported by Howard et al. (2000) in various *Capsicum annuum* cultivars.

β -carotene content. The red color of ripening pepper comes from the accumulation of carotenoid in the pericarp of pepper fruits (Markus et al., 1999). Compared with the GM stage, as can be expected, an $\approx$ 6-fold

increment in β -carotene content could be observed in the RR stage (Fig. 1D) as was previously reported by Gnaifed et al. (2001) and Navarro et al. (2006). However, we observed higher β -carotene content ranging

Table 1. Free sugar contents in pepper fruits at three ripening stages.

Cultivars	Ripening stages	Free sugar content (mg·g ⁻¹ FW)			
		Fructose	Glucose	Sucrose	Total
21-Segi	Green mature	6.2 ± 0.3 a ^{z,y}	10.1 ± 0.1 a	5.6 ± 0.1 a	21.9 ± 0.5 a
	Breaker	9.2 ± 1.3 b	11.3 ± 1.2 a	14.7 ± 1.8 b	35.2 ± 4.3 b
	Red ripe	25.7 ± 1.2 c	23.0 ± 0.9 b	N/D ^x	48.8 ± 2.2 c
Baerotta	Green mature	5.3 ± 0.3 a	8.4 ± 0.1 a	9.7 ± 0.3 a	23.4 ± 0.1 a
	Breaker	12.7 ± 0.1 b	13.8 ± 0.2 b	15.3 ± 0.8 b	41.8 ± 0.9 b
	Red ripe	23.3 ± 0.4 c	22.6 ± 0.5 c	N/D	45.9 ± 0.8 c
Muhanjilju	Green mature	7.0 ± 0.2 a	8.9 ± 0.3 a	4.1 ± 0.3 a	19.9 ± 0.4 a
	Breaker	12.9 ± 0.4 b	13.1 ± 0.6 b	11.1 ± 0.1 b	37.0 ± 1.0 b
	Red ripe	20.0 ± 0.5 c	17.4 ± 1.2 c	N/D	37.3 ± 1.6 c
PR-Mujeokplus	Green mature	5.8 ± 0.3 a	7.4 ± 0.2 a	6.3 ± 0.4 a	19.5 ± 0.5 a
	Breaker	13.0 ± 0.4 b	12.2 ± 0.6 b	11.0 ± 0.2 b	36.2 ± 1.2 b
	Red ripe	22.2 ± 0.5 c	19.6 ± 0.8 c	N/D	41.8 ± 1.3 b
Superbigarim	Green mature	4.1 ± 0.1 a	6.3 ± 0.0 a	8.6 ± 0.3 a	19.0 ± 0.6 a
	Breaker	4.6 ± 0.8 a	5.7 ± 1.1 a	20.6 ± 2.2 b	30.7 ± 4.0 b
	Red ripe	17.3 ± 0.6 b	15.7 ± 1.4 b	N/D	33.0 ± 2.0 b

^zValues are mean ± SD of three replicates in fresh weight basis.

^yDifferent letters among ripening stages within a cultivar indicate significant difference by Duncan's multiple range test at $P \leq 0.05$.

^xNot detected.

FW = fresh weight.

Table 2. Vitamin E isomer contents in pepper fruits at three ripening stages.

Cultivars	Ripening stages	Vitamin E isomer content (mg·kg ⁻¹ DW)				Total vitamin E (mg·kg ⁻¹ DW)
		α -tocopherol	β -tocopherol	γ -tocopherol	δ -tocopherol	
21-Segi	Green mature	74.1 ± 0.8 a ^{z,y}	0.7 ± 0.1 a	9.5 ± 0.2 a	2.1 ± 0.0 c	86.4 ± 1.1 a
	Breaker	95.4 ± 3.6 b	0.8 ± 0.1 a	16.4 ± 0.9 b	1.0 ± 0.2 b	113.5 ± 4.6 b
	Red ripe	138.5 ± 4.1 c	1.7 ± 0.1 b	18.3 ± 0.8 c	0.8 ± 0.1 a	159.2 ± 4.8 c
Baerotta	Green mature	67.3 ± 1.3 a	0.8 ± 0.1 a	22.5 ± 0.3 a	2.2 ± 0.1 b	92.8 ± 1.3 a
	Breaker	118.4 ± 1.3 b	0.9 ± 0.1 b	31.4 ± 2.3 b	1.5 ± 0.1 a	152.2 ± 2.6 b
	Red ripe	176.5 ± 2.9 c	2.2 ± 0.1 c	38.7 ± 1.3 c	1.3 ± 0.1 a	218.8 ± 4.4 c
Muhanjilju	Green mature	68.1 ± 2.1 a	0.8 ± 0.1 a	26.0 ± 0.8 a	1.7 ± 0.3 b	96.6 ± 2.7 a
	Breaker	116.0 ± 7.8 b	1.4 ± 0.1 b	44.6 ± 2.6 b	1.0 ± 0.1 a	163.0 ± 10.4 b
	Red ripe	149.4 ± 3.1 c	3.2 ± 0.4 c	43.6 ± 1.4 b	1.3 ± 0.2 a	197.5 ± 4.8 c
PR-Mujeokplus	Green mature	55.2 ± 2.2 a	0.6 ± 0.1 a	17.5 ± 0.5 a	1.6 ± 0.4 a	74.9 ± 2.3 a
	Breaker	113.3 ± 2.0 b	1.3 ± 0.2 b	31.0 ± 0.8 b	1.8 ± 0.3 a	147.5 ± 3.1 b
	Red ripe	161.4 ± 5.6 c	2.1 ± 0.2 c	35.0 ± 2.0 c	1.6 ± 0.2 a	200.1 ± 7.8 c
Superbigarim	Green mature	65.1 ± 1.4 a	0.9 ± 0.1 a	17.7 ± 0.3 a	1.7 ± 0.1 b	85.4 ± 1.4 a
	Breaker	75.5 ± 3.3 b	0.9 ± 0.1 a	30.5 ± 1.3 b	0.9 ± 0.1 a	107.8 ± 4.5 b
	Red ripe	218.4 ± 3.5 c	4.1 ± 0.2 b	32.6 ± 0.8 b	1.2 ± 0.3 a	256.4 ± 4.7 c

^zValues are mean ± SD of three replicates in dry weight basis.

^yDifferent letters among ripening stages within a cultivar indicate significant difference by Duncan's multiple range test at $P \leq 0.05$.

DW = dry weight.

Table 3. Squalene and phytosterols content (mg·kg⁻¹ DW) in pepper fruits at three ripening stages.

Cultivars	Ripening stages	Squalene	Phytosterols			Total phytosterols
			Campesterol	Stigmasterol	β -sitosterol	
21-Segi	Green mature	10.0 ± 0.2 b ^{z,y}	40.7 ± 0.1 c	28.0 ± 0.3 c	135.6 ± 0.8 c	204.3 ± 1.0 c
	Breaker	12.6 ± 0.8 c	19.0 ± 0.9 a	19.2 ± 0.6 a	69.5 ± 2.4 a	107.7 ± 3.9 a
	Red ripe	5.6 ± 0.1 a	22.3 ± 0.8 b	22.6 ± 1.1 b	89.1 ± 3.6 b	134.0 ± 5.5 b
Baerotta	Green mature	21.9 ± 0.2 c	44.8 ± 1.3 c	39.5 ± 0.6 c	153.5 ± 4.4 c	237.7 ± 6.2 c
	Breaker	18.0 ± 0.8 b	32.5 ± 1.3 b	30.8 ± 2.3 b	122.3 ± 9.5 b	185.7 ± 13.1 b
	Red ripe	9.4 ± 0.1 a	22.9 ± 0.8 a	23.1 ± 0.9 a	95.2 ± 3.7 a	141.3 ± 5.4 a
Muhanjilju	Green mature	19.6 ± 0.7 b	52.5 ± 2.6 c	32.9 ± 1.5 b	180.3 ± 10.1 c	265.7 ± 13.8 c
	Breaker	24.5 ± 1.1 c	20.5 ± 1.8 a	25.1 ± 1.5 a	82.2 ± 7.6 a	127.8 ± 10.8 a
	Red ripe	9.1 ± 0.1 a	25.3 ± 0.6 b	26.5 ± 0.8 a	105.1 ± 3.5 b	156.9 ± 4.8 b
PR-Mujeokplus	Green mature	15.0 ± 0.4 b	58.3 ± 5.6 b	42.4 ± 2.7 b	162.9 ± 18.1 b	263.7 ± 26.3 b
	Breaker	19.9 ± 0.2 c	36.7 ± 1.4 a	41.2 ± 0.9 b	124.9 ± 9.5 a	202.8 ± 7.3 a
	Red ripe	8.5 ± 0.4 a	30.8 ± 0.8 a	32.8 ± 1.3 a	110.8 ± 20.5 a	174.3 ± 4.5 a
Superbigarim	Green mature	10.4 ± 0.8 a	26.9 ± 0.5 c	26.5 ± 0.4 a	91.8 ± 3.2 a	145.1 ± 3.9 a
	Breaker	15.7 ± 0.7 a	24.9 ± 1.3 b	32.9 ± 1.2 c	88.9 ± 4.8 a	145.6 ± 7.3 a
	Red ripe	6.4 ± 0.0 a	22.2 ± 0.8 a	28.8 ± 0.6 b	93.6 ± 3.0 a	144.5 ± 4.3 a

^zValues are mean ± SD of three replicates in dry weight basis.

^yDifferent letters among ripening stages within a cultivar indicate significant difference by Duncan's multiple range test at $P \leq 0.05$.

DW = dry weight.

from 198.4 to 291.8 mg·kg⁻¹ in the GM stage to 511.8 to 1291.3 mg·kg⁻¹ in the RR stage than in red pepper (Navarro et al., 2006) and sweet pepper (Deepa et al., 2007) cultivars. In contrast, Deepa et al. (2007) observed some wide variation (≈13-fold increases) among the maturity stages in sweet pepper cultivars. More prominent β-carotene accumulation could be observed between the BR and RR stages in that β-carotene content was low in the GM stage, exhibited a slight increase during the BR stage, and increased significantly during the RR stage. Although we observed the same ripening-dependent pattern of increase in all five tested cultivars, the magnitude of the increase between the GM and RR stages was highest in ‘21-Segi’ (596%) with a 596% increase in the RR stage (1183.1 mg·kg⁻¹ compared with 198.4 mg·kg⁻¹ in the GM stage) followed by ‘Superbigarim’ (544%), ‘PR-Mujeokplus’ (518%), ‘Muhanjilju’ (381%), and ‘Baerotta’ (203%) indicating that both the cultivars and ripening stages are determinants of the β-carotene in red peppers.

Free sugar content. The content of free sugars is strongly related to the sweet taste of peppers and consequently affects the marketability of harvested peppers (Soh et al., 2011). Both fructose and glucose contents significantly increased as ripening progressed in four of the tested cultivars; the exception was ‘Superbigarim’, which showed no statistically significant difference between the GM and BR stages (Table 1). Sucrose content showed a different accumulation pattern in that it increased from GR to BR and then decreased to a level below the detection limit in the RR stage (Table 1). The absence of sucrose in fully ripened pepper fruits is consistent with reports by Hubbard and Pharr (1992) and Navarro et al. (2006), who suggested there was a degradation of sucrose and subsequent increases in hexose sugars. Similar to the findings of Navarro et al. (2006), the total free sugar concentration increased significantly as peppers became fully RR in all of the cultivars with ‘21-Segi’ having the highest total free sugar content (48.8 mg·g⁻¹) among the cultivars.

Vitamin E content. The analysis of vitamin E isomers in pepper fruits showed the presence of four tocopherol isomers, α-, β-, γ- and δ-tocopherols, but the absence of any tocotrienol form of vitamin E. Among the four tocopherol isomers, α-tocopherol was present in the highest quantity (55.2 to 218.4 mg·kg⁻¹), accounting for more than 70% of total vitamin E regardless of ripening stage and cultivar (Table 2), which suggests its high health potential value because α-tocopherol is a well-known natural antioxidant (Ohkatsu et al., 2001) and the quantity in this study was higher than in other vegetables such as lady finger (12.8 mg·kg⁻¹), pumpkin (12.7 mg·kg⁻¹), and carrot (29.9 mg·kg⁻¹) (Ching and Mohamed, 2001). In all of the tested cultivars, total vitamin E content increased significantly as the pepper fruit ripened from GM to BR and RR, mainly owing to an increase in α-tocopherol. Other forms of tocopherol isomers exhibited inconsistent

Table 4. Changes in fatty acid composition (%) in pepper fruits at three ripening stages.

Cultivars	Ripening stages	Lauric (C12:0)	Myristic (C14:0)	Palmitic (C16:0)	Palmitoleic (C16:1)	Stearic (C18:0)	Oleic (C18:1)	Linoleic (C18:2)	Linolenic (C18:3)	Arachidic (C20:0)	Behenic (C22:0)	UFA ^y	
												MUFA ^x	Total
21-Segi	Green mature	N/D ^v	0.32 a	20.35 a	0.48 a	3.91 c	4.37 a	61.03 c	9.01 a	0.22 a	0.30 b	4.85 a	70.04 c
	Breaker	0.07 a ^{u,t}	0.83 b	21.90 b	0.52 a	3.35 b	6.64 b	56.94 b	9.40 a	0.16 a	0.20 a	7.16 b	66.34 b
Baerotta	Red ripe	0.24 b	1.62 c	22.34 b	0.66 b	2.88 a	6.88 b	52.53 a	12.48 b	0.18 a	0.20 a	7.54 c	65.00 a
	Green mature	N/D	0.29 a	21.99 a	0.45 a	3.31 a	4.13 a	60.54 b	8.70 a	0.23 a	0.36 a	4.58 b	69.24 b
Muhanjilju	Breaker	0.09 a	0.81 b	22.40 a	0.55 a	3.18 a	2.67 b	60.02 b	9.88 b	0.18 a	0.22 a	3.22 a	69.90 b
	Red ripe	0.25 b	1.27 c	21.91 a	0.73 b	3.16 a	6.35 c	54.69 a	11.28 c	0.20 a	0.18 a	7.08 c	73.05 a
PR-Mujeokplus	Green mature	N/D	0.33 a	20.31 a	0.34 a	3.01 b	4.24 a	63.22 b	8.12 a	0.18 b	0.27 b	4.58 a	71.34 c
	Breaker	0.06 a	0.74 b	21.40 ab	0.46 b	2.61 a	5.98 b	61.15 b	7.39 a	0.05 a	0.14 a	6.45 b	68.54 b
Superbigarim	Red ripe	0.22 b	1.58 c	22.95 b	0.68 c	2.52 a	7.68 c	51.61 a	12.54 b	0.10 a	0.15 a	8.36 c	64.16 a
	Green mature	N/D	0.36 a	21.57 a	0.41 a	3.29 b	3.97 a	61.31 a	8.52 b	0.22 b	0.35 b	4.38 a	69.83 b
Superbigarim	Breaker	0.10 a	0.99 b	21.95 a	0.48 b	2.59 a	6.11 b	60.21 a	7.27 a	0.09 a	0.19 a	6.59 c	67.49 a
	Red ripe	0.26 b	1.52 c	21.77 a	0.53 c	2.45 a	5.74 c	58.81 a	8.64 b	0.11 a	0.17 a	6.27 b	67.45 a
Superbigarim	Green mature	N/D	0.37 a	25.69 a	0.56 a	3.86 a	3.86 a	55.01 b	10.04 a	0.21 b	0.33 b	4.42 a	65.05 a
	Breaker	0.06 a	0.68 b	25.51 a	0.48 a	2.79 b	6.63 c	54.51 b	9.07 a	0.11 a	0.17 a	7.11 c	63.58 a
Superbigarim	Red ripe	0.53 b	1.96 c	25.38 a	0.83 b	2.47 a	5.39 b	47.10 a	16.00 b	0.13 a	0.20 a	6.22 b	63.11 a

^zSFA = saturated fatty acids; detected.

^yUFA = unsaturated fatty acids.

^xMUFA = monounsaturated fatty acids.

^wPUFA = polyunsaturated fatty acids.

^vNot detected.

^uValues are mean of three replicates.

^tDifferent letters among ripening stages within a cultivar indicate significant difference by Duncan's multiple range test at $P \leq 0.05$.

patterns of change during ripening and could not affect changes in total vitamin E content because of their lower quantity compared with α -tocopherol. Our results are consistent with those of Osuna-Garcia et al. (1998) and Menichini et al. (2009), who observed higher vitamin E levels in mature paprika and hot peppers, respectively. Among the tested cultivars, Superbigarim had the highest ripening-dependent increase; its total vitamin E content increased to 256.4 mg·kg⁻¹ in the RR stage compared with 85.4 mg·kg⁻¹ in GM, corresponding to a 300% increase.

Squalene and phytosterols contents. Squalene, a lipophilic phytonutrient with proliferative and serum cholesterol-lowering effects (Khor and Chieng, 1997), has rarely been studied in peppers. In our study, squalene content in pepper fruit ranged from 5.6 to 24.5 mg·kg⁻¹ throughout the ripening stages, showing slight increases in BR and then decreasing again in the RR stage in most cultivars (Table 3). 'Baerotta' was an exceptional case, having a continuously decreasing squalene content from GM (21.9 mg·kg⁻¹) to BR (18.0 mg·kg⁻¹) and RR (9.4 mg·kg⁻¹). Among the three major phytosterols (campesterol, stigmasterol, and β -sitosterol) that were analyzed in this study, β -sitosterol ranged from 69.5 to 180.3 mg·kg⁻¹ and was the major phytosterol in all cultivars and ripening stages (Table 3). Different cultivars exhibited different ripening-dependent changes in total phytosterol content with '21-Segi' and 'Muhanjilju' having their lowest levels in the BR stage, 'Baerotta' and 'PR-Mujeokplus' showing continuous decreases, and 'Superbigarim' showing no changes during ripening. These cultivar-specific and ripening-dependent patterns of change in total phytosterol content were observed in all three analyzed phytosterols. This is the first report to our knowledge that generalizes ripening-dependent changes in squalene and major phytosterols in red peppers. Although our results suggest that all types of phytosterols in pepper fruits at the GM stage can decrease as ripening progresses, further careful study of phytosterols using a larger number of pepper cultivars is required.

Fatty acid composition. Under our experimental conditions, 10 fatty acids, including six saturated fatty acids (SFA), two monounsaturated fatty acids, and two polyunsaturated fatty acids, out of 37 targeted fatty acids, were detected in pepper fruits (Table 4). Three major fatty acids were linoleic (47.10% to 63.22%), palmitic (20.31% to 25.69%), and linolenic (7.27% to 16.00%) acids. The high compositional ratio of polyunsaturated fatty acids (linoleic and linolenic acid) in pepper fruits is useful for human health because these fatty acids are responsible for reducing the rates of cardiovascular disease and type 2 diabetes (Willett, 2007). Linoleic acid, which was the most abundant in pepper fruits (47.10% to 63.22%), was highest in the GM stage and then gradually decreased during ripening. Most cultivars showed similar decreasing patterns, with 'Muhanjilju' showing the greatest decrease (from 63.2%

in GM to 51.6% in RR) and 'PR-Mujeokplus' showing a relatively smaller decrease (from 61.3% in GM to 58.8% in RR). The second major fatty acid was palmitic acid, which did not exhibit any changes during ripening. In contrast, linolenic, myristic, and oleic acids exhibited statistically significant increases in the RR stage compared with the GM stage. Lauric acid, which could not be detected in the GM stage, showed continuous increases during ripening. However, ripening-dependent changes in SFA and unsaturated fatty acids were only statistically significant in '21-Segi'

and 'Muhanjilju'. So further study is required to clarify these unusual changes of fatty acids in pepper cultivars because this report seems to be the first one describing ripening-related fatty acid changes in pepper.

Capsaicinoid content. Two major capsaicinoids, capsaicin and dihydrocapsaicin, were analyzed in this study, and different cultivars exhibited three different patterns of ripening-dependent changes. The first pattern included '21-Segi', 'PR-Mujeokplus', and 'Superbigarim', which had their highest capsaicinoid contents in the GM stage with

Fig. 2. Changes in (A) capsaicin and (B) dihydrocapsaicin contents in pepper fruits at three ripening stages. Values are mean $\pm$ SD of three replicates. Different letters within the same cultivar indicate statistically significant difference at $P \leq 0.05$ by Duncan's multiple range test.

Fig. 3. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging capacity of pepper fruits at three ripening stages. Values are mean $\pm$ SD of three replicates. Different letters within the same cultivar indicate statistically significant difference at $P \leq 0.05$ by Duncan's multiple range test.

Table 5. Correlation coefficients among phytonutrient contents and antioxidant activity of pepper fruits.

Attributes	Capsaicinoid	Vitamin E	Squalene	Campesterol	Stigmasterol	β -sitosterol	Total phytosterol	Total phenol	Total flavonoid	β -carotene	AA ^z
Vitamin C	-0.091	0.781**	-0.294*	-0.697**	-0.496**	-0.606**	-0.636**	0.709**	0.093	0.768**	0.610**
Capsaicinoid		-0.334*	-0.263	-0.206	-0.566**	-0.295*	-0.321*	0.023	0.361*	-0.174	-0.135
Vitamin E			-0.399**	-0.573**	-0.318*	-0.439**	-0.471**	0.672**	-0.132	0.773**	0.814**
Squalene				0.362*	0.448**	0.371*	0.393**	-0.395**	0.139	-0.642**	-0.584**
Campesterol					0.770**	0.964**	0.982**	-0.756**	-0.273	-0.486**	-0.447**
Stigmasterol						0.730**	0.805**	-0.651**	-0.358*	-0.317*	-0.247
β -sitosterol							0.991**	-0.598**	-0.254	-0.396**	-0.367*
Total phytosterol								-0.667**	-0.283	-0.422**	-0.384**
Total phenol									0.313*	0.684**	0.595**
Total flavonoid										-0.101	0.122
β -carotene											0.772**

^zAA = antioxidant activity.*, **Correlation is significant at $P \leq 0.05$ and $P \leq 0.01$, respectively.

gradual decreases when peppers reached the RR stage (Fig. 2). In 'Muhanjilju' (case 2), the highest capsaicinoid levels were observed in the intermediate BR stage, and in 'Baerotta' (case 3), the highest capsaicinoid content was found in the RR stage. Both capsaicin (Fig. 2A) and dihydrocapsaicin (Fig. 2B) showed similar trends, and consequently total capsaicinoid content showed the same pattern. These results suggest that the ripening-dependent accumulation of capsaicinoids is highly dependent on the cultivar. Many studies have examined the accumulation of capsaicinoids (Conforti et al., 2007; Deepa et al., 2007), and peroxidase has been suggested as a major factor of decline (Gnayfeed et al., 2001). Decreases in capsaicinoids after a certain physiological period are thought to be the result of changes in peroxidase activity, which may control the synthesis of capsaicinoids (Contreras-Padilla and Yahia, 1998; Estrada et al., 2000). As was suggested by Zewdie and Bosland (2000), capsaicinoid content in pepper may be readily affected by various environmental conditions and developmental stages. The fact that '21-Segi', which showed the highest capsaicinoid contents among tested cultivars, exhibited the same capsaicinoid accumulation pattern as 'PR-Mujeokplus' and 'Superbigarim' that showed relatively low capsaicinoid contents suggests no direct relationship between maturity-dependent changes and the level of capsaicinoid accumulation. In our experiments, only five cultivars were used and consequently more cultivar-specific studies on capsaicinoid changes during the process of ripening are needed by using a higher number of cultivars as well as by relating with other physiological aspects of fruit ripening.

Antioxidant activity. The antioxidant activity of pepper fruits as measured by DPPH radical scavenging activity increased significantly with the advance of ripening in all cultivars (Fig. 3). Among the five cultivars, IC₅₀ value that represents the concentration required to obtain a 50% antioxidant capacity ranged from 19.16 to 23.19 mg·mL⁻¹ in the GM stage to 10.64 to 14.39 mg·mL⁻¹ in the RR stage and 'Superbigarim' exhibited the largest ripening-dependent increase in antioxidant activity with an IC₅₀ value of 23.19 mg·mL⁻¹ in GM compared with

10.64 mg·mL⁻¹ in RR. These results are consistent with those of Ghasemnezhad et al. (2011) and Howard et al. (2000), who reported higher antioxidant activity levels in various mature pepper species. However, ripening-dependent changes in antioxidant activity were lower than compared with the previously described phytochemicals that were analyzed.

Correlations between phytonutrients and antioxidant activity. As was described previously, the phytonutrient contents of pepper fruits were affected by ripening. Antioxidant activity, which is an indicator of the overall health benefits of pepper fruits, also changed during ripening. To address questions about ripening-independent relationships between antioxidant activity and fluctuating phytonutrient contents, correlations between IC₅₀ and all phytonutrients in all ripening stages in all tested cultivars were evaluated. We found strong positive correlations between antioxidant activity and vitamin E ($r = 0.814^{**}$), β -carotene ($r = 0.772^{**}$), vitamin C ($r = 0.610^{**}$), and total phenol ($r = 0.595^{**}$) throughout the entire ripening process (Table 5). These phytonutrients are all well-known antioxidant compounds, and these results are consistent with previous reports that described the antioxidant effects of various fruits and vegetables (Aires et al., 2011; Alvarez-Parrilla et al., 2011; Naguib et al., 2012). In addition, strong positive correlations among the listed phytonutrients were observed (Table 5), indicating that the accumulation of these compounds was the major cause of the increase in antioxidant activity in pepper fruits with the advance of ripening. In contrast, similar to the results of Alvarez-Parrilla et al. (2011) and Materska and Perucka (2005), no correlations were observed between antioxidant activity and capsaicinoid content, possibly because of the low antioxidant capacity of capsaicinoid compounds or the low capsaicinoid contents in our pepper samples. Similarly, campesterol, stigmasterol, β -sitosterol, and total flavonoid showed either negative or no correlation with antioxidant activity. Strong positive correlations were found among three tested phytosterols, campesterol, stigmasterol, and β -sitosterol, possibly because these compounds share the same biosynthetic pathway (Piironen et al., 2000).

The present study identified changes in various phytochemicals and antioxidant activity in pepper fruits in different ripening stages. Vitamin C, total phenol, vitamin E, total free sugar, β -carotene, linolenic acid, and antioxidant activity increased as peppers matured and entered the RR stage. Phytosterols (campesterol, stigmasterol, and β -sitosterol) and linoleic acid were highest in the GM stage, and palmitic acid, squalene, and total flavonoid were highest in the BR stage. In contrast, capsaicinoids showed three different patterns of change depending on the cultivar examined. All of these results suggest that phytonutrients in peppers are, although affected by ripening in different ways depending on the nature of compounds, follow a similar pattern of ripening-dependent changes regardless of cultivar. Among phytonutrients vitamin C, vitamin E, β -carotene, and total phenol content showed the strongest positive correlations with antioxidant activity regardless of ripening of pepper fruits. These findings may also provide additional phytonutrient-related information to customers consuming peppers as a fresh vegetable or as a spicy powder that are produced with pepper fruits harvested at green and fully red stages, respectively.

Literature Cited

- Aires, A., C. Fernandes, R. Carvalho, R.N. Bennett, M.J. Saavedra, and E.A.S. Rosa. 2011. Seasonal effects on bioactive compounds and antioxidant capacity of six economically important *Brassica* vegetables. *Molecules* 16: 6816–6832.
- Alvarez-Parrilla, E., L.A. De La Rosa, R. Amarowicz, and F. Shahidi. 2011. Antioxidant activity of fresh and processed Jalapeno and Serrano peppers. *J. Agr. Food Chem.* 59:163–173.
- Bae, H., G.K. Jayaprakasha, J. Jifon, and B.S. Patil. 2012. Variation of antioxidant activity and the levels of bioactive compounds in lipophilic and hydrophilic extract from hot pepper (*Capsicum* spp.) cultivars. *Food Chem.* 134:1912–1918.
- Balasundram, N., K. Sundram, and S. Samman. 2006. Phenolic compounds in plants and agro-industrial by-products: Antioxidant activity, occurrence, and potential uses. *Food Chem.* 99:191–203.
- Bhandari, S.R., S. Basnet, K.H. Chung, K.-H. Ryu, and Y.-S. Lee. 2012. Comparisons of nutritional and phytochemical property of genetically modified CMV-resistant red pepper and

- its parental cultivar. *Hort. Environ. Biotechnol.* 53:151–157.
- Burton, G.W. and M.G. Traber. 1990. Vitamin E: Antioxidant activity, biokinetics, and bioavailability. *Annu. Rev. Nutr.* 10:357–382.
- Ching, L.S. and S. Mohamed. 2001. Alpha-tocopherol content in 62 edible tropical plants. *J. Agr. Food Chem.* 49:3101–3105.
- Cisneros-Pineda, O., L.W. Torres-Tapia, L.C. Gutierrez-Pacheco, F. Contreras-Martin, T. Gonzalez-Estrada, and S.R. Peraza-Sanchez. 2007. Capsaicinoids quantification in chilli peppers cultivated in the state of Yucatan, Mexico. *Food Chem.* 104:1755–1760.
- Conforti, F., G.A. Statti, and F. Menichini. 2007. Chemical and biological variability of hot pepper fruits (*Capsicum annuum* var. *acuminatum* L.) in relation to maturity stage. *Food Chem.* 102:1096–1104.
- Contreras-Padilla, M. and E.M. Yahia. 1998. Changes in capsaicinoids during development, maturation, and senescence of chile peppers and relation with peroxidase activity. *J. Agr. Food Chem.* 46:2075–2079.
- Czeczot, H. 2000. Biological activities of flavonoids—A review. *Pol. J. Food Nutr. Sci.* 950:3–13.
- Deepa, N., C. Kaur, B. George, B. Singh, and H.C. Kapoor. 2007. Antioxidant constituents in some sweet pepper (*Capsicum annuum* L.) genotypes during maturity. *LWT-Food Sci. Technol.* 40: 121–129.
- Estrada, B., M.A. Bernal, J. Diaz, F. Pomar, and F. Merino. 2000. Fruit development in *Capsicum annuum*: Changes in capsaicin, lignin, free phenolics and peroxidase patterns. *J. Agr. Food Chem.* 48:6234–6239.
- Garces-Claver, A., R. Gil-Ortega, A. Alvarez-Fernandez, and M.S. Arnedo-Andres. 2007. Inheritance of capsaicin and dihydrocapsaicin, determined by HPLC-ESI/MS, in an intraspecific cross of *Capsicum annuum* L. *J. Agr. Food Chem.* 55:6951–6957.
- Ghasemnezhad, M., M. Sherafati, and G.A. Payvast. 2011. Variation in phenolic compounds, ascorbic acid and antioxidant activity of five coloured bell pepper (*Capsicum annuum*) fruits at two different harvest times. *J. Funct. Foods* 3:44–49.
- Gnayfeed, M.H., H.G. Daoood, P.A. Biacs, and C.F. Alcaraz. 2001. Content of bioactive compounds in pungent spice red pepper (paprika) as affected by ripening and genotype. *J. Sci. Food Agr.* 81:1580–1585.
- Guerra, M., R. Magdaleno, and P.A. Casquero. 2011. Effect of site and storage conditions on quality of industrial fresh pepper. *Sci. Hort.* 130:141–145.
- Guil-Guerrero, J.L., C. Martinez-Guirado, M.M. Rebolloso-Fuentes, and A. Carrique-Perez. 2006. Nutrient composition and antioxidant activity of 10 pepper (*Capsicum annuum*) varieties. *Eur. Food Res. Technol.* 224:1–9.
- Halliwell, B. 1996. Antioxidants in human health and disease. *Annu. Rev. Nutr.* 16:33–50.
- Hargrove, R.L., T.D. Etherton, T.A. Pearson, E.H. Harrison, and P.M. Kris-Etherton. 2001. Low-fat and high-monounsaturated fat diets decrease human low-density lipoprotein oxidative susceptibility *in vitro*. *J. Nutr.* 131: 1758–1763.
- Howard, L.R., S.T. Talcott, C.H. Brenes, and B. Villalon. 2000. Changes in phytochemical and antioxidant activity of selected pepper cultivars (*Capsicum* species) as influenced by maturity. *J. Agr. Food Chem.* 48:1713–1720.
- Hubbard, N.L. and D.M. Pharr. 1992. Developmental changes in carbohydrate concentration and activities of sucrose metabolizing enzymes in fruits of two *Capsicum annuum* L. genotypes. *Plant Sci.* 86:33–39.
- Khor, H.T. and D.Y. Chieng. 1997. Effect of squalene, tocotrienols and α -tocopherol supplementations in the diet on serum and liver lipids in the hamster. *Nutr. Res.* 17:475–483.
- Kim, S., J.-B. Park, and I.-K. Hwang. 2002. Quality attributes of various varieties of Korean red pepper powders (*Capsicum annuum* L.) and color stability during sunlight exposure. *J. Food Sci.* 67:2957–2961.
- Koleva, I.I., T.A. van Beek, J.P.H. Linssen, A. de Groot, and L.N. Evstatieva. 2002. Screening of plant extracts for antioxidant activity: A comparative study on three testing methods. *Phytochem. Anal.* 13:8–17.
- Korean Statistical Information Service. 2012. Agriculture, forestry and fishery survey. Daejeon, Republic of Korea.
- Lee, S.K. and A.A. Kader. 2000. Preharvest and postharvest factors influencing vitamin C content of horticultural crops. *Postharvest Biol. Technol.* 20:207–220.
- Lee, Y., L.R. Howard, and B. Villalon. 1995. Flavonoids and antioxidant activity of fresh pepper (*Capsicum annuum*) cultivars. *J. Food Sci.* 60:473–476.
- Manach, C., A. Mazur, and A. Scalbert. 2005. Polyphenols and prevention of cardiovascular diseases. *Curr. Opin. Lipidol.* 16:77–84.
- Marangoni, F. and A. Poli. 2010. Phytosterols and cardiovascular health. *Pharmacol. Res.* 61: 193–199.
- Marin, A., J.S. Rubio, V. Martinez, and M.I. Gil. 2009. Antioxidant compounds in green and red peppers as affected by irrigation frequency, salinity and nutrient solution composition. *J. Sci. Food Agr.* 89:1352–1359.
- Marinova, D., F. Ribarova, and M. Atanassova. 2005. Total phenolics and total flavonoids in Bulgarian fruits and vegetables. *J. Univ. Chem. Tech. Met.* 40:255–260.
- Markus, F., H.G. Daoood, J. Kapitany, and P.A. Biacs. 1999. Change in the carotenoid and antioxidant content of spice red pepper (paprika) as a function of ripening and some technological factors. *J. Agr. Food Chem.* 47:100–107.
- Materska, M. and I. Perucka. 2005. Antioxidant activity of the main phenolic compounds isolated from hot pepper fruit (*Capsicum annuum* L.). *J. Agr. Food Chem.* 53:1750–1756.
- Menichini, F., R. Tundis, M. Bonesi, M.R. Loizzo, F. Conforti, G. Statti, B. De Cindio, P.J. Houghton, and F. Menichini. 2009. The influence of fruit ripening on the phytochemical content and biological activity of *Capsicum chinense* Jacq. cv Habanero. *Food Chem.* 114:553–560.
- Middleton, E., Jr., C. Kandaswami, and T.C. Theoharides. 2000. The effects of plant flavonoids on mammalian cells: Implications for inflammation, heart disease, and cancer. *Pharmacol. Rev.* 52:673–751.
- Miliauskas, G., P.R. Venskutonis, and T.A. van Beek. 2004. Screening of radical scavenging activity of some medicinal and aromatic plant extracts. *Food Chem.* 85:231–237.
- Mori, A., S. Lehmann, J. O’Kelly, T. Kumagai, J.C. Desmond, M. Pervan, W.H. McBride, M. Kizaki, and H.P. Koeffler. 2006. Capsaicin, a component of red peppers, inhibits the growth of androgen-independent, p53 mutant prostate cancer cells. *Cancer Res.* 66:3222–3229.
- Nagata, M. and I. Yamashita. 1992. Simple method for simultaneous determination of chlorophyll and carotenoids in tomato fruits. *J. Japan Food Sci. Technol.* 39:925–928.
- Naguib, A.E.M.M., F.K. El-Baz, Z.A. Salama, H.A.E.B. Hanaa, H.F. Ali, and A.A. Gaafar. 2012. Enhancement of phenolics, flavonoids and glucosinolates of Broccoli (*Brassica oleracea*, var. *Italica*) as antioxidants in response to organic and bio-organic fertilizers. *J. Saudi Soc. Agr. Sci.* 11:135–142.
- Navarro, J.M., P. Flores, C. Garrido, and V. Martinez. 2006. Changes in the contents of antioxidant compounds in pepper fruits at different ripening stages, as affected by salinity. *Food Chem.* 96:66–73.
- Ohkatsu, Y., T. Kajiyama, and Y. Arai. 2001. Antioxidant activities of tocopherols. *Polym. Degrad. Stabil.* 72:303–311.
- Osuna-Garcia, J.A., M.M. Wall, and C.A. Waddell. 1998. Endogenous levels of tocopherols and ascorbic acid during fruit ripening of new Mexican-type chile (*Capsicum annuum* L.) cultivars. *J. Agr. Food Chem.* 46:5093–5096.
- Piironen, V., D.G. Lindsay, T.A. Miettinen, J. Toivo, and A.M. Lampi. 2000. Plant sterols: Biosynthesis, biological function and their importance to human nutrition. *J. Sci. Food Agr.* 80:939–966.
- Rietjens, I.M.C.M., M.G. Boersma, L. de Haan, B. Spenkelenk, H.M. Awad, N.H.P. Cnubben, J.J. van Zanden, H. van der Woude, G.M. Alink, and J.H. Koeman. 2002. The pro-oxidant chemistry of the natural antioxidants vitamin C, vitamin E, carotenoids and flavonoids. *Environ. Toxicol. Pharmacol.* 11:321–333.
- Singleton, V.L. and J.A. Rossi, Jr. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Amer. J. Enol. Viticult.* 16:144–158.
- Soh, J.-W., K.-Y. Choi, Y.-B. Lee, and S.-Y. Nam. 2011. Analysis of component factors concerned in taste of Korean hot pepper by sensory evaluation. *J. Bio-Environ. Contr.* 20:297–303.
- Topuz, A. and F. Ozdemir. 2007. Assessment of carotenoids, capsaicinoids and ascorbic acid composition of some selected pepper cultivars (*Capsicum annuum* L.) grown in Turkey. *J. Food Compos. Anal.* 20:596–602.
- van Rensburg, S.J., W.M.U. Daniels, J.M. van Zyl, and J.J.F. Taljaard. 2000. A comparative study of the effects of cholesterol, beta-sitosterol, beta-sitosterol glucoside, dehydroepiandrosterone sulphate and melatonin on *in vitro* lipid peroxidation. *Metab. Brain Dis.* 15:257–265.
- Wahyuni, Y., A.-R. Ballester, E. Sundarmonowati, R.J. Bino, and A.G. Bovy. 2011. Metabolite biodiversity in pepper (*Capsicum*) fruits of thirty-two diverse accessions: Variation in health-related compounds and implications for breeding. *Phytochem.* 72:1358–1370.
- Weryszko-Chmielewska, E. and Z. Michalojc. 2011. Anatomical traits of sweet pepper (*Capsicum annuum* L.) fruit. *Acta Agrobot.* 64:181–188.
- Willett, W.C. 2007. The role of dietary n-6 fatty acids in the prevention of cardiovascular disease. *J. Cardiovasc. Med.* 8:S42–S45.
- Zewdie, Y. and P.W. Bosland. 2000. Evaluation of genotype, environment and genotype-by-environment interaction for capsaicinoids in *Capsicum annuum* L. *Euphytica* 111:185–190.
- Zhuang, Y., L. Chen, L. Sun, and J. Cao. 2012. Bioactive characteristics and antioxidant activities of nine peppers. *J. Funct. Foods* 4:331–338.