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Original Research Article

Ascorbic acid, capsaicinoid, and flavonoid aglycone concentrations as a function of fruit maturity stage in greenhouse-grown peppers

Haejin Bae [a], G.K. Jayaprakasha [a], Kevin Crosby [a], Kil Sun Yoo [a], Daniel I. Leskovar [a,c], John Jifon [a,b,*], Bhimanagouda S. Patil [a,**]

[a] Vegetable and Fruit Improvement Center, Department of Horticultural Sciences, Texas A&M University, College Station, TX 77845, USA
[b] Texas A&M AgriLife Research Center, Weslaco, TX 78596, USA
[c] Texas A&M AgriLife Research Center, Uvalde, TX 78801, USA

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ABSTRACT

Bioactive compounds in plant-derived foods provide essential human health benefits. However, the content and types of bioactive compounds may vary based on genetic and environmental factors. The present study examined the impacts of cultivar, fruit maturity stage (mature versus immature), and growing season (2008, 2009) on the concentration of bioactive compounds in diverse pungent and non-pungent peppers. Significant interactions were observed among cultivars, maturity stages, and growing seasons. Mature peppers generally had the highest content of ascorbic acid (782.0–2305.3 μg/g FW in 2008 and 693.5–2817.2 μg/g FW in 2009), and capsaicinoids (115.5–338.9 μg/g FW in 2008 and 93.8–326.3 μg/g FW in 2009) compared to immature peppers. Paprika-type peppers generally had the highest contents of ascorbic acid and flavonoids especially in mature fruits, while capsaicinoids were higher in all mature stage peppers. Flavonoid concentrations varied considerably depending on cultivar and maturity stage. Total phenolics were also significantly higher in mature fruits compared to immature peppers in both years. A positive correlation between total phenolics and DPPH radical scavenging activity was observed. This indicates that the health beneficial components in peppers could also vary as a function of cultivar, fruit developmental stage and production season. The genetic variability in bioactive compounds found in this study constitutes a useful genetic base for improving the nutrient quality of peppers.

1. Introduction

Ample evidence of the link between diet and health has stimulated intense research on the bioactive properties of foods (Patil et al., 2012). Fruits, vegetables, and spices are rich in diverse bioactive compounds such as ascorbic acid, capsaicinoids, flavonoids, carotenoids, phenolics and capsiates, which are linked with promotion of health and wellbeing (Archer and Jones, 2002; Bhattacharya et al., 2010; Jayaprakasha and Rao, 2011; Jayaprakasha et al., 2012b; Johnson and de Mejia, 2011; Materska and Perucka, 2005; Palozza et al., 2011; Patil et al., 2009). While crop genetics clearly plays a significant role in the content and diversity of bioactive compounds (Navarro et al., 2006), environmental conditions and agronomic practices can interact with genetics and other factors to alter the bioactive properties of foods (Jifon et al., 2012).

However, only a few studies have reported on the impacts of environmental factors and cultivation practices on the content of bioactive compounds. Deepa et al. (2007) reported higher levels of ascorbic acid and capsaicinoids in mature than in immature peppers. Flavonoid content has been reported to decrease as peppers mature (Marin et al., 2004). Peppers grown in a greenhouse have also shown less variation in the content of bioactive compounds compared to field-grown peppers because of the controlled environmental and growing conditions such as light, temperature, nutrients, and irrigation (Russo and Howard, 2002). In this study, we have investigated the effects of cultivar type, fruit maturity stage, and growing season on the content of ascorbic acid, capsaicinoids, flavonoids, and total phenolics, as well as DPPH radical scavenging activity in greenhouse-grown peppers in 2008 and 2009.

* Corresponding author at: Texas A&M AgriLife Research, 2415 E Highway 83, Texas A&M University, Weslaco, TX 78596, USA. Tel.: +1 956 968 5585; fax: +1 956 969 5020.
** Corresponding author at: Vegetable and Fruit Improvement Center, Department of Horticultural Sciences, 2119, Texas A&M University, College Station, TX 77845, USA. Tel.: +1 979 845 2743; fax: +1 979 862 4522.
E-mail addresses: jljifon@tamu.edu (J. Jifon), b-patil@tamu.edu (B.S. Patil).

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2. Materials and methods

2.1. Pepper cultivars and growing conditions

This study was conducted during the spring-summer season (April–August) of 2008 and 2009 in a sunlit, ventilated greenhouse at the Vegetable and Fruit Improvement Center, Texas A&M University (College Station, TX, USA). Six-week-old seedlings of eight pepper cultivars (non-pungent and pungent types) were transplanted into 12 L plastic pots containing soilless media (Pro Mix® BX, Premier Horticulture Inc., Quakertown, PA, USA). The pungent pepper types are cayenne (*C. annuum* L. cv. 'CA408′ and 'Mesilla'), jalapeño (*C. annuum* L. cv. 'Ixtapa'), and serrano (*C. annuum* L. cv. 'Tuxtlas'); the non-pungent types are Habanero (*Capsicum. chinense* L. cv. 'TAM Mild Habanero' or 'TMH'), jalapeño (*C. annuum* L. cv. 'TAM Mild Jalapeño' or 'TMJ'), and paprika (*C. annuum* L. cv. 'PA137′ and 'B58′). Six plants of each cultivar were used for each of four replications in each growing season. The experimental design was completely randomized with eight cultivars, and peppers were harvested at two maturity stages. After transplanting, green peppers were harvested at 56 days, and red (except TMH yellow peppers) were harvested at 113 days. Plants were watered at least once per day, depending on the evaporative demand using an automatic drip irrigation system, and fertigated two times per week with a complete water-soluble fertilizer (4.5N–9.9P–6.3K, Peter's Corp., St. Louis, MO, USA) after transplanting. During flowering and fruit set development, plants were fertigated two times per week with a 10N–4.4P–8.3K nutrient solution. Average day/night greenhouse temperatures during the study period were 34.4/23.3 °C in 2008 and 36.1/25.0 °C in 2009. Average daily solar radiation intensities during 2008 and 2009 were 20.78 and 20.40 MJ/m^2 respectively (TexasET, 2008–2009). Immature fruits were defined as firm fruits that had reached full commercial size, but flesh color was still completely green. Fruits were considered mature when flesh surface had turned completely red, orange or yellow, depending on cultivar. The duration from transplanting to immature and mature stages varied with cultivar. Immature peppers (green) were harvested in July at 56 days after transplanting and mature (fully red ripe except TMH at yellow color) peppers were harvested in August at 56 days after green fruit harvest in both years. About 18 random fruits considered as sub-samples were collected from within a plant. Whole fruits, excluding stalks, were sliced, macerated, and stored at −80 °C until analysis. All samples were analyzed for bioactive compounds within one month after harvest. All results are expressed on a fresh weight (FW) basis.

2.2. Ascorbic acid and capsaicinoid analysis

Each pepper sample (5 g) was homogenized with 40 mL of solvent mixture, 3% meta-phosphoric acid and ethanol (8:2), for extraction of ascorbic acid and capsaicinoids. Metaphosphoric acid and ethanol (200 proof) solutions were purchased from Sigma–Aldrich (St. Louis, MO, USA). The homogenate was sonicated for 30 min, centrifuged, filtered through a 0.45 μm membrane and analyzed by HPLC. Calibration curves for ascorbic acid (Mallinckrodt, Paris, KY, USA), capsaicin (purity 95%), and dihydrocapsaicin (purity ≥90%) (Sigma–Aldrich, St. Louis, MO, USA) were prepared through serial dilution of different concentrations of standards and area. The Perkin Elmer HPLC system (Salem, MA, USA) included a LC-250 B pump, a Nelson 900 autosampler, and diode array detector 235C. HPLC analyses were performed on a C_{18} Gemini (Phenomenex, Torrance, CA, USA) HPLC column (250 mm × 4.6 mm i.d., 5 μm particle size) with gradient mobile phase of solvent A (0.03 mM of phosphoric acid in water) and solvent B (methanol). The gradient program was as follows: 0% B (0–5 min), 0–100% B (5–12 min), 100% B (12–15 min), and 100–0% B (15–20 min) with a flow rate of 1 mL/min. The column was equilibrated for 5 min before the next injection. Ascorbic acid and capsaicinoids were detected at 254 and 282 nm, respectively (Bae et al., 2013). The HPLC chromatograms of ascorbic acid and capsaicinoids are shown in Fig. 1. Peak identification was confirmed by spiking samples with known concentrations of standards. The limits of detection (LOD), limit of quantification (LOQ), recovery, and linear dynamic range of the curves for quantification of ascorbic acid and capsaicinoids were presented in our recent publication (Bae et al., 2013).

2.3. Analysis of flavonoids

Five flavonoids were analyzed by HPLC as described (Bae et al., 2012). Quercetin (purity ≥98%), luteolin (purity ≥98%), kaempferol (purity ≥90%), apigenin (purity ≥95%), myricetin (purity ≥96%) were obtained from Sigma–Aldrich (St. Louis, MO, USA) and used for generating the calibration curves. Each pepper sample (5 g) was homogenized with 40 mL of ethanol and *N*,*N*-dimethylformamide (Sigma–Aldrich, St. Louis, MO, USA) for extraction of flavonoids. The homogenate was further extracted by shaking for 3 h at room temperature and filtered. Six millilitres of the filtrate was treated with 3 ml of 3 N HCl, and heated at 95 °C in a water bath for 1 h for the conversion of flavonoid glycosides to aglycones. Flavonoids were separated on a C_{18} Gemini column (250 mm × 4.6 mm i.d., 5 μm) with the following gradient program: solvent A (0.03 M of phosphoric acid in water) and solvent B (methanol). The gradient elution was as follows: a linear gradient of 40–100% B (0–10 min), 100% B (12–15 min), and a linear gradient of 100–40% B (15–20 min). Flavonoids were detected at 360 nm with a flow rate of 1 mL/min. Each peak was confirmed by spiking selected samples with a known amount of each flavonoid standard according to our recently published method (Bae et al., 2013).

Typical HPLC chromatograms of flavonoids and samples are presented in Fig. 2. All method validation parameters including LOD, LOQ, and linear dynamic range of the curves for each compound were presented in recent publication (Bae et al., 2013).

2.4. Total phenolics and DPPH (2,2-diphenyl-1-picrylhydrazyl) assay

Total phenolics were evaluated by the Folin–Ciocalteu method using (+)-catechin (Sigma–Aldrich, St. Louis, MO, USA) according to our publication (Jayaprakasha and Patil, 2007). Total phenolics and DPPH (Sigma–Aldrich, St. Louis, MO, USA) radical scavenging activities were measured from samples prepared for flavonoid analysis. An aliquot (100 μL) of the sample extract was transferred to a test tube and brought up to volume (10 mL) using distilled water; 500 μL of the diluted Folin–Ciocalteu reagent (1:1) (Biomedicals, Illkirch, France) was added to each test tube and incubated at room temperature for 10 min, then 1 mL of saturated sodium carbonate was added into the tubes and incubated for a further 20 min. Absorbance of the ensuing blue color was measured at 760 nm. Total phenolics were expressed as mg of catechin equivalents/g of FW. Catechin standard was used for measuring total phenolics due to stability and high purity.

The DPPH assays were conducted according to a modified method (Jayaprakasha et al., 2008). The DPPH solution (40 mg) was prepared with 1000 mL of methanol. Ascorbic acid was dissolved in 3% meta-phosphoric acid and used as a standard (150 μg/mL). Samples prepared for the analysis of flavonoids were also used for DPPH assays. Sample aliquots (10 μL) and standard ascorbic acid were pipetted into a 96-well microplate. The volume of each well was adjusted to 100 μL with MeOH, and then 180 μL of DPPH solution was added. The color change was monitored by absorbance at 515 nm for 30 min using a KC-4 microplate reader