



A profile of physicochemical and antioxidant changes during fruit growth for the utilisation of unripe apples

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ABSTRACT

The physicochemicals associated with fruit quality, antioxidant activities and changes in polyphenol composition during Fuji apple growth were investigated from the 25th to 105th day after full bloom day (DAFB). Three kinds of antioxidant assays, including oxygen radical absorbing capacity (ORAC) were executed, and polyphenol composition were examined using HPLC. The change in weight of the Fuji apples during fruit growth showed a typical sigmoidal curve. Both the rise of the soluble solid content and the drop of the titratable acidity appeared to be more significant ($p < 0.05$) after the 85th DAFB. Concurrently, the antioxidant activities and the polyphenol content decreased ($p < 0.05$) rapidly after the same date. Accordingly, a profitable thinning date can be chosen around the 85th DAFB to utilise dropped unripe apples as resources of antioxidants. Furthermore, the antioxidant activities of Fuji apples may be highly affected by the chlorogenic acid content.

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1. Introduction

Apples are one of the most cultivated and consumed tree fruits in the world. They not only have a lovely flavour but also contain functional compounds such as polyphenols (Schieber, Petra, & Reinhold, 2001). Polyphenols have been reported to exert a variety of biological activities such as free radical scavenging activity (Adil, Çetin, Yener, & Bayındır, 2007; Sudha, Baskaran, & Leelavathi, 2007), metal chelation (Chien, Sheu, Huang, & Su, 2007), anti-allergic activity (Akiyama et al., 2005; Kojima et al., 2000), anti-arteriosclerosis activity (D'Angelo et al., 2007; Stefania et al., 2007). Unripe apples discarded in the orchards by thinning or natural drop, accounted for 20–30% of the total apple production in Korea in 2007 (Zheng, Hwang, & Chung, 2009), but only a small part of them are utilised as ingredients for animal feed or fertiliser. The composition of polyphenols from unripe apples heavily depends upon their variety and growth stage (Akiyama et al., 2005; Wu et al., 2007). In particular, the total phenolic content (TPC) (Renard, Dupont, & Guillermin, 2007), proanthocyanidin (Akiyama et al., 2005), and flavonoid (Mohamed, Anton, Linus, & Alexander, 2001) content of unripe apples were higher than those in ripe apples. Some agro-industry by-products, such as hulls, peels, and pomaces were reviewed for the utilisation of their phenolic

compounds (Balasundram, Sundram, & Samman, 2006). It is also very favourable for growers to utilise thinned apples as bioactive resources. Accordingly, we have investigated the antioxidant activities and changes in polyphenol composition in Fuji apples, along with some physicochemical quality changes during fruit growth.

2. Materials and methods

2.1. Plant materials

Apples (*Malus pumila* cv. Fuji) were picked from different trees within the same rows in the orchard of Kyungpook National University, Korea, at 25, 45, 65, 85, 105, 125, 145, 165, and 185 days after full bloom day (DAFB, April 11) in 2009.

2.2. Reagents and instruments

Folin–Ciocalteu phenol reagent, DPPH (1,1-diphenyl-2-picrylhydrazyl), (±)-catechin hydrate, chlorogenic acid, caffeic acid, *p*-coumaric acid, phloretin, and phloridzin were purchased from Sigma Co. (St. Louis, MO, USA). Procyanidin B1 was purchased from Extrasynthese Co. (Lyon, France). Quercetin-3-galactoside and quercetin-3-glucoside were obtained from Plantech Co. (Reading, UK). In addition, all other organic solvents used were of analytical grade and purchased from Merck (Darmstadt, Germany), except for the HPLC grade solvents which were purchased from J.T. Baker (Phillipsburg, NJ). Other chemicals were purchased from Duksan Co. (Seoul, Korea).

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A UV–Visible spectrophotometer (UV 1601 PC, Shimadzu, Co., Kyoto, Japan), a HPLC (LC-10A, Shimadzu, Co., Kyoto, Japan) associated with the UV–Visible detector (SPD-10A, Shimadzu, Co., Kyoto, Japan), and a Victor 3 1420 multilabel counter (PerkinElmer Inc., Boston, MA, USA) were used in the study.

2.3. Apple sample preparation

For the physicochemical analyses, 100 g of fresh apples were blanched at 80 °C for 3 min (Buckow, Weiss, & Dietrich, 2009) and crushed with a home blender. The complex was centrifuged (250 g, 5 min), and the supernatant was collected and used. To prepare the sample solutions of antioxidant activities and polyphenol analysis, 100 mL of ethanol (two times) was added to the blanched apples (100 g), crushed, centrifuged, and filled in a 250 mL of volumetric flask. The HPLC sample solution was filtered through a 0.45 µm filter.

2.4. Physicochemical determination

2.4.1. Apple growth indices

The fresh fruit weight, diameter, water content, pH, acidity, and soluble solids were measured as apple growth indices. The weight and diameter of 10 intact selected apples were measured using a METTLER TOLEDO digital balance (B3002DR, Schwarzenbasch, Switzerland) and an ALLTRADE dial caliper (Long beach, CA, USA), respectively. The pH and soluble solids were estimated by a METTLER TOLEDO pH meter (MP 220, Schwarzenbasch, Switzerland) and a digital refractometer (ATAGO RX-5000, Houston, TX, USA), respectively. The moisture content and the titratable acidity were also determined during apple growth by the oven drying method and neutralisation method, respectively.

2.4.2. Reducing sugar content (RSC)

The RSC was determined by the dinitrosalicylic acid (DNS) method (Miller, 1959) with some modifications, and the contents were expressed as mg of glucose equivalents per gram of fresh sample weight. Briefly, 500 µL of DNS reagent and 150 µL of sample were added to the test tube, boiled at 98 °C for 5 min, and cooled to room temperature, and the absorbance was measured at 550 nm.

2.5. Antioxidant determination

2.5.1. Oxygen radical absorbance capacity (ORAC)

Determination of oxygen radical absorbance capacity (ORAC) was performed on a Victor 3 multilabel counter (Model 1420, PerkinElmer Inc., Boston, MA, USA), according to the method of Ou, Hampsch-Woodill, and Prior (2001) with some modifications. Approximately 120 µL of fluorescein (200 nm) and 20 µL of diluted sample, phosphate buffer saline (PBS) as blank, or standard (trolox, 20–100 µM) were placed in each well of a 96 well-plate. After 60 µL of 2,2'-azobis(2-methylpropionamidine) dihydrochloride (AAPH, 221 mM) were added into the wells, the fluorescence was measured every 80 s (excitation 488 nm, emission 520 nm). ORAC values were expressed as mmol trolox equivalents per gram of fresh sample weight (mmol TE/g).

2.5.2. DPPH radical scavenging activity

The scavenging activities for DPPH radicals were determined according to Lim, Lim, and Tee (2007). The activities were expressed in mmol trolox equivalents per gram of fresh sample weight (mmol TE/g).

2.5.3. Ferric reducing antioxidant power (FRAP)

The FRAP assay was performed using TPTZ (2,4,6-tripyridyl-S-triazine) solution according to the methods (Benzie & Strain, 1996) with modifications (Shahrokh et al., 2008). The working solution was prepared by mixing 25 mL of acetate buffer (pH 3.6), 2.5 mL of TPTZ solution (10 mM), and 2.5 mL of FeCl₃·6H₂O solution (20 mM), and warmed at 37 °C just before every determination. And then, 175 µL of this working solution was mixed with 25 µL of the sample solution. After incubated at 37 °C for 30 min in the dark, the absorbance of the sample solution was measured at 590 nm. Results are expressed in mmol trolox equivalents per gram of fresh sample weight (mmol TE/g).

2.5.4. Total phenolic content (TPC)

The total phenolic content (TPC) was measured by the spectrometric method using Folin–Ciocalteu reagent (Singleton, Orthofer, & Lamuela-Raventos, 1999).

2.5.5. Total flavonoid content (TFC)

The total flavonoid content (TFC) was measured by the method of Jia, Tang, and Wu (1999) with modifications (Moreno, Isla, Sampietro, & Vattuone, 2000). The TFC was expressed in mg of rutin equivalents per 100 g of sample (mg RE/100 g). One millilitre of the sample solution was added to test tube containing 0.1 mL of 10% aluminium nitrate, 0.1 mL of 1 M potassium acetate, and 3.8 mL of ethanol. After 40 min at room temperature, the absorbance was measured at 415 nm. TFC was expressed in mg rutin equivalents per 100 g of fresh sample weight (mg RE/100 g).

2.5.6. Polyphenol composition

The investigation of polyphenol composition was performed on an HPLC (LC-10A, Shimadzu, Co., Kyoto, Japan) equipped with a UV–Visible detector (SPD-10A, Shimadzu, Co., Kyoto, Japan) at 290 nm using an ODS-HG-5 column (Develosil, 150 × 4.6 mm, i.d.) and a security guard C₁₈ ODS (4 × 3.0 mm, i.d.). The mobile phase consisted of 2% (v/v) acetic acid in water (eluent A) and 0.5% (v/v) acetic acid and 49.5% (v/v) acetonitrile in water (eluent B). The gradient was programmed as follows: 12–25% B (0–15 min), 25–35% B (15–25 min), 35–55% B (25–50 min), 55–65% B (50–60 min), and 65–12% B (60–70 min). The flow rate and injection volume were 1.0 mL/min and 20 µL, respectively.

2.6. Statistical analyses

All examinations were executed in triplicate, and the values are reported as mean ± standard deviation. Statistical analyses were performed by the Statistical Analysis System (SAS version 9.1, SAS Institute Inc. NC, US, 2003). Values were evaluated by the analysis of variance (ANOVA), followed by Duncan's multiple range tests ($p < 0.05$).

3. Results and discussion

3.1. Physicochemical changes during the growth of the fruits

The physicochemical changes of Fuji apples during fruit growth (e.g., fruit weight and diameter, moisture content, acidity, and reducing sugar content) were investigated. The physicochemical changing profile in Fuji apples during the fruit growth is very important in choosing the harvesting date because harvest maturity significantly affects the quality and shelf-life of ripe apples (Kulkarni & Aradhya, 2005).

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