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Evaluation of physiochemical and antioxidant activity changes during fruit on-tree ripening for the potential values of unripe peaches

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ABSTRACT

The changes of physiochemicals associated with fruit quality, ascorbic acid and polyphenol content, and antioxidant properties during the last month on-tree ripening were investigated. Identification and quantification of ascorbic acid and polyphenols were performed using HPLC method. The antioxidant activities were evaluated by means of several *in vitro* assays, such as the β-carotene/linoleate model system, ABTS, DPPH and FRAP. The fruit growth resulted in statistically increased content of TSS and the reductions of the acidity ($p < 0.05$). Concurrently, the ascorbic acid and polyphenol content as well as antioxidant activities decreased ($p < 0.05$) dramatically over the ripening period. The results indicated that dropped unripe peaches are excellent sources of bioactive and antioxidant compounds, and can be explored for their health promoting values in food products. Furthermore, the antioxidant activity may be highly affected by hydroxycinnamates (chlorogenic and neo-chlorogenic acid) and flavan-3-ols contents.

1. Introduction

In recent years, overwhelming epidemiological and clinical investigations demonstrated that regular consumption of fruits and their relevant products can promote an excellent state of health in humans, lower the risk of several chronic diseases such as cardiovascular disease, cancer, obesity and diabetes, and even slow the aging process (Borbalán et al., 2003; Kris-Etherton et al., 2002). These chemoprotective effects related to fruit consumption can be largely attributed to a wide variety of potential phytochemicals presented in plants, of which phenolics constitute the greatest proportion (Olsson et al., 2004; Sun et al., 2002). Phenolic antioxidants were believed to contribute to health benefits through several possible mechanisms, by scavenging free radical species, chelating transition metals, reducing peroxides and stimulating antioxidant enzyme defense (Rice-Evans et al., 1996). As a consequence, there has been a renewed interest in the evaluation of the phenolics and their antioxidant properties of the most common species.

Peach [*Prunus persica* (L.) Batsch] fruit, which belongs to the Rosaceae family, is indigenous to China over 3000 years and now cultivated widely in appropriate climates around the world. Peach enriches with an excellent source of nutritive components which contribute significantly to human nutrition and is considered to be one of the most important and popular commodity consumed worldwide due to its delicious flavor and attractive appearance. Moreover, peach has been reported to contain a variety of polyphenols, such as chlorogenic acid, neochlorogenic acid, catechin, epicatechin and derivatives of cyaniding and quercetin (Tomás-Barberán et al., 2001). It has been proved that the phenolic profile and antioxidant activity of fruits are affected by maturity, genotype, horticultural practices, geographic origin, growing season, postharvest storage conditions and processing procedure (Díaz-Mula et al., 2009; Dragovic-Uzelac et al., 2007; Falguera et al., 2012; Leong and Oey, 2012).

To date, unripe peaches discarded in the orchards by thinning or natural drop, accounted for a significant percentage of the total peach production in China, but a large part of them were discarded as waste. The composition of polyphenols from unripe peaches heavily depended upon their variety and growth stage (Scordino et al., 2012). In particular, unripe fruits were regarded as rich sources of polyphenols, such as phenolics, proanthocyanidin and flavonoid (Dragovic-Uzelac et al., 2007). Some agro-industry by-products, such as seeds, peels and pomaces were reviewed for the utilization of their phenolic compounds (Bocco et al., 1998; Lee and Wrolstad, 2004). It was also very favorable for growers to utilize thinned or naturally dropped peaches as bioactive resources. Therefore, our present investigation determined the changes of ascorbic acid and polyphenol contents as well as antioxidant activities of five commercial peach cultivars from China during on-tree ripening.

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2. Material and methods

2.1. Chemicals

Gallic acid, Folin–Ciocalteu's reagent, L-ascorbic acid, β-carotene, dithiothreitol (DTT), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis-(3-ethyl-benzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ), 6-hydroxy-2,5,7,8-tetramethyl chroman-2-carboxylic acid (Trolox, a hydrophilic derivative of tocopherol), linoleic acid, Tween 20 were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). The phenolic standards of protocatechuic acid, chlorogenic acid (5-O-caffeoylquinic acid), neo-chlorogenic acid (3-O-caffeoylquinic acid), (+)-catechin and quercetin-3-rutinoside were from Fluka–Sigma–Aldrich® (St. Louis, MO, USA). Methanol, acetone, chloroform, acetic and formic acid of HPLC grade were supplied by Merck (Darmstadt, Germany). Potassium persulfate, ferrous sulfate and other chemicals used were of standard analytical grade. Deionized and distilled water was used throughout.

2.2. Peach fruit sampling

Peach fruits [*P. persica* (L.) Batsch] were collected in an experimental orchard in Beijing, including five cultivars of 'Yuhualu' (cv. Yh), 'Dahonghua' (cv. Dh), 'Hujingmilu'(cv. Hj), 'Fenghuayulu' (cv. Fh) and 'Wulingyulu' (cv. Wl) represented different types of peach according to relative mature period. Cv. Yh, cv. Dh and cv. Hj are early-maturing cultivars; while cv. Fh and cv. Wl are late-maturing cultivars.

The experiment involved fifteen or twenty trees with the same growth vigor and tree age for each cultivar. The same trees were selected for samplings during the whole picking time. Peaches were harvested for each cultivar at 7-day intervals during the last month of ripening. The four picking dates were named as *t*1, *t*2, *t*3, *t*4 and the last date (*t*4) coincided with eating quality (commercial maturation). For each sampling date and each cultivar 80 similar fruits (size, color and with absence of any defect) were manually picked and transferred immediately to the laboratory. Each cultivar was divided into two equal parts. One part was used for measurement of fruit weight, firmness, color, total soluble solids (TSS) and titratable acidity (TA) immediately after picking. And the other part was used for determination of ascorbic acid and phenolic content and antioxidant activities. The samples of this part were freeze-dried at −50 °C under 5 mtorr (9.67×10^{-5} psi) vacuum for 48 h in a freeze drier. Then the freeze-dried material was ground using a pestle and mortar and stored at −40 °C until extraction.

Each fruit was weighed on a precision scale to 0.01 g confidence level. The skin color was measured in the blushed and un-blushed sides of fruit using a CR-400 chromameter (Minolta, Osaka, Japan) and expressed in Commission Internationale de l'Eclairage L^*, a^*, and b^* (CIELAB) color space coordinates. The skin was removed on four sides of each fruit, and firmness was measured four times on each fruit using a digital penetrometer (GY-4, Zhejiang, China) with an 11 mm tip. TSS content was measured by crushing the flesh and transferring the intact juice to a digital refractometer, model WM-7 (Atago, Tokyo, Japan). TA was determined using an acid-base titration method. Five grams of homogenate were diluted with 25 mL of distilled H_2O and titrated with 0.1 mol/L NaOH solution up to pH 8.1. The results were expressed as percent malic acid.

2.3. Peach sample preparation

Lyophilized peach were extracted following conditions previously selected (Pérez-Jiménez and Saura-Calixto, 2005) with slight modifications. One gram of the sample was placed in a capped centrifuge tube and 20 mL of acidic methanol–water (50:50, v/v, pH 2) were added, after which the tube was vortexed for 1 min and shaken at room temperature for 1 h. The tube was then centrifuged at 12,000 × *g* and the supernatant was recovered. Then 20 mL of 70% aqueous acetone was added to the residue, followed by stirring, shaking and centrifugation. The supernatants were combined and diluted to a final volume of 20 mL with water and used to analysis of phenolics, flavonoids and antioxidant activity.

2.4. Determination of total phenolics (TPs)

The extracts were diluted with the same solvent, used for extraction to a suitable concentration for analysis. Total phenolic content in the extracts was determined using the Folin–Ciocalteu method (Singleton and Rossi, 1965) with some modifications. The TPs content was calculated from the calibration curve prepared with gallic acid, and the results were expressed as gallic acid equivalent in milligrams per kilogram of fresh weight (mg GAE kg^{-1} FW).

2.5. Determination of total flavonoids (TFs)

The total flavonoids content was estimated using a colorimetric method (Bakar et al., 2009). Rutin was chosen as the standard. The TFs was determined at 510 nm with spectrophotometer. The data were expressed as mg rutin equivalents in one kilogram of fresh weight (mg RE kg^{-1} FW).

2.6. Extract and analysis of L-ascorbic acid (vitamin C)

L-ascorbic acid was extracted as the method (Hernández et al., 2006) with some modifications. Peach samples (1.0 g) were extracted with 10 mL cold NaH_2PO_4 buffer solution (20 mM, pH 2.1). The mixture was sonicated for 15 min and centrifuged at 12,000 × *g* for 20 min at 4 °C. This procedure was repeated two times and the resulting supernatants were collected and brought to final volume of 20 mL. Immediately, 800 μL of the filtered extract (0.2 cm cellulose syringe filter) was added to 200 μL of DTT (20 g/L) as a reducing agent, left for 2 h before HPLC analysis (Gracea et al., 2014). L-ascorbic acid was determined by RP-HPLC method using a C18 column (Shim–pack VP-ODS 15 cm× 4.6 mm ID, 5 μm, Shimadzu Co., Japan) where 10 μL was injected for each sample. The elution was conducted isocratically using a mixture of 90% formic acid (0.1%) and 10% methanol at a flow rate of 0.8 mL/min. The quantification was performed from the peak areas recorded at 245 nm with reference to the calibration curve obtained with L-ascorbic acid reference.

2.7. Identification and quantification of phenolic compounds

HPLC analysis was performed using Shimadzu LC-20AT pumps, SPD-M20A diode array detection, and chromatographic separations were performed on a C18 column (Shim–pack VP-ODS 15 cm × 4.6 mm ID, 5 μm, Shimadzu Co., Japan). The mobile phase consisted of 1% (v/v) acetic acid in water (eluent A) and methanol (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). Operating conditions were as follows: column temperature, 35 °C, injection volume, 10 μL and UV-diode array detection at 280 nm (phenolic compound) and 370 nm (flavonoids). Phenolic compounds in the samples were identified by comparing their relative retention times and UV spectra with those of authentic compounds and were detected using an external standard method.