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Evaluation and comparison of vitamin C, phenolic compounds, antioxidant properties and metal chelating activity of pulp and peel from selected peach cultivars

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ABSTRACT

This study determined the total phenolic and flavonoid contents, ascorbic acid and phenolic composition and evaluated antioxidant properties and metal chelating activity of five peach cultivars, separately for pulp and peel. Identification and quantification of ascorbic acid and phenolic compounds were performed using HPLC method. The antioxidant and metal chelating activities are evaluated by means of several *in vitro* methods, such as the β-carotene/linoleate model system, radical scavenging, reducing power, and iron and copper ion chelation. The results showed that the peach peels have the highest antioxidant capacities, reflecting their highest content of total phenolics, flavonoids, ascorbic acid and phenolic compounds. In addition, the predominant phenolic compounds in peaches was found to be chlorogenic acid, followed by neo-chlorogenic acid, (+)-catechin, quercetin-3-rutinoside and protocatechuic acid. The results indicated that peach peels are good sources of bioactive and antioxidant compounds, and can be explored for their health promoting values in food products.

1. Introduction

In recent years, overwhelming epidemiological studies have demonstrated that there is a significant positive relationship between fruit consumption and reduced risk of certain chronic diseases (Borbalán, Zorro, Guillén, & Barroso, 2003; Kris-Etherton et al., 2002). These chemoprotective effects related to fruit consumption can be largely attributed to the optimal combination of essential vitamins, minerals, fiber and bioactive phytochemicals such as alkaloids, carotenoids and polyphenols (Saura-Calixto & Goñi, 2006). Antioxidant polyphenolics, by virtue of their hydrogen and electron donating abilities and metal chelating effects, exhibit a wide range of biological properties including anti-inflammatory, anti-microbial, anti-allergenicity, cardioprotective and vasodilatory actions (Middleton, Kandaswami, & Theoharides, 2000; Puupponen-Pimiä et al., 2001).

Peach [*Prunus persica* (L.) Batsch] has been reported to contain a variety of phenolics, such as chlorogenic acid, neochlorogenic acid, catechin, epicatechin and derivatives of cyaniding and quercetin (Tomás-Barberán et al., 2001). Composition and concentrations of these phytochemicals vary according to maturity, genotype, horticultural practices, geographic origin, postharvest storage conditions and processing procedure (Dragovic-Uzelac, Levaj, Mrkic, Bursac, & Boras, 2007; Falguera et al., 2012; Kalinowska, Bielawska & Lewandowska-Siwkiewic, Priebe, & Lewandowski, 2014).

Because peach is a seasonal fruit, a significant percentage of fresh peaches are processed for products, such as juices, purees and canned slices that are popular worldwide (Ávila & Silva, 1999; Brenna et al., 2000; Campbell & Padilla-Zakour, 2013). Peel is a major by-product of such industrial processing; peach peels are not currently used commercially, but are discarded as waste that create disposal, sanitation and environmental problems. However, according to previous studies, peel has been found to be an excellent source of phytochemicals, such as polyphenols, carotenoids, vitamin C and it exhibited good antioxidant properties (Albishi, John, Al-Khalifa, & Shahidi, 2013; Contreras-Calderón, Calderón-Jaimes, Guerra-Hernández, & García-Villanova, 2011). The external location of phenolic compounds is believed to associate with their main natural function: protection against environmental stress and

Abbreviations: TPs, total phenolics; TFs, total flavonoids; AA, ascorbic acid; TEAC, Trolox equivalent antioxidant capacity; DPPH, 2,2-diphenyl-1-picrylhydrazyl; FRAP, ferric reducing antioxidant power; CUPRAC, cupric ion (Cu^{2+}) reducing assay; GAE, gallic acid equivalents; RE, rutin equivalents.

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pathogens (Treutter, 2005). For this reason, it seems that peach peel may be a rich source of natural antioxidants and worthy of further study.

Though considerable work has been done with regard to antioxidants and polyphenols of peach, very few reports are available with regard to peach peel. In this present study, we for the first time report the phenolic and vitamin C composition, *in vitro* antioxidant potencies and metal chelating activity of pulp and peel for five peach cultivars which are commercially important in China.

2. Material and methods

2.1. Standard and 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, neocuproine 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). Other chemicals used were of standard analytical grade.

2.2. Fruits samples and extraction procedures

Ripened fresh peach fruits were collected successively from 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) cultured traditionally in China, representing different types of peach according to their relative mature period. Among these peach species tested, cv. Yh, cv. Dh and cv. Hj are early-maturing cultivars; while cv. Fh and cv. Wl are late-maturing cultivars. For each cultivar 80 similar fruits (size, color and with absence of any defect) were manually picked and transferred immediately to the laboratory. Upon harvest ten fruits per cultivar were washed with deionized water and towel dried and the peel and pulp was separated and scraped using a knife and then immediately plunged into liquid nitrogen. All samples were freeze-dried at −50 °C under 5 mtorr (9.67×10^{-5} psi) vacuum for 48 h in a freeze drier. The freeze-dried material was ground using a pestle and mortar and stored at −20 °C.

Powdered peach tissues were extracted following conditions previously selected (Pérez-Jiménez & 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 shaken at room temperature for 1 h. The tube was then centrifuged at 3500 rotation per minute (rpm) and the supernatant was recovered. Then 20 mL of 70% aqueous acetone were added to the residue, followed by stirring, shaking and centrifugation. The methanolic and acetonic extracts were combined and evaporated to dryness under vacuum at 30 °C. Phenolic compounds extracted were reconstituted in 10 mL of deionized water and stored at −20 °C until used before 24 h.

2.3. Determination of total phenolics content

Total phenolics content in the extracts was determined using the Folin–Ciocalteu method (Singleton & Rossi, 1965) with some modifications. A 1.5 mL amount of 10-fold diluted Folin-Ciocalteu reagent was added to 150 μL of the sample extract. After 5 min, 1.5 mL of sodium carbonate (6%, w/v) was added, mixed and incubated in water bath at 75 °C for 10 min and rapidly cooled in ice bath. Absorbance was measured at 725 nm using a spectrophotometer. The TPs contents were expressed as gallic acid equivalent in milligrams per 100 g of fresh weight (mg GAE/100 g FW).

2.4. Determination of flavonoids content

The total flavonoids content was estimated using a colorimetric method (Bakar, Mohamed, Rahmat, & Fry, 2009). Rutin was chosen as the standard and the results were expressed as mg rutin equivalents in 100 g of fresh weight (mg RE/100 g FW).

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

L-ascorbic acid was extracted as method (Hernández, Gloria Lobo, & González, 2006) with some modifications. Freeze-dried peach samples (1.0 g) were extracted with 10 mL cold NaH_2PO_4 buffer solution (20 mM, pH 2.1, containing 1 mM EDTA). The resulting mixture was sonicated for 15 min and centrifuged at 4000 rpm 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 at 4 °C before analysis. Precautions were made to avoid light exposure throughout the whole procedure. L-ascorbic acid was determined by 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.

2.6. 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 methods.

2.7. Antioxidant assay using a β-carotene/linoleate model system

Antioxidant activity was determined also using the β-carotene bleaching test with some modifications (Conforti, Statti, Tundis, Loizzo, & Menichini, 2007). Briefly, 1 mL of β-carotene solution (0.2 mg/mL in chloroform) was added to 0.05 mL of linoleic acid and 0.4 mL of Tween 20. After evaporation of chloroform and dilution with water, 3 mL of the emulsion was transferred into test tubes containing 0.2 mL of samples. The tubes were then gently shaken and placed at 50 °C in water bath for 120 min. The absorbance of the samples was measured at 470 nm against a blank,