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Short communication

Phenolic profiles and antioxidant activity of litchi pulp of different cultivars cultivated in Southern China

Ruifen Zhang [a,1], Qingshuai Zeng [a,b,1], Yuanyuan Deng [a], Mingwei Zhang [a,b,*], Zhencheng Wei [a], Yan Zhang [a], Xiaojun Tang [a]

[a] Bio-Technical Research Institute, Guangdong Academy of Agricultural Sciences, Guangzhou 510610, PR China
[b] Department of Food Science and Technology, Huazhong Agricultural University, Wuhan 430070, PR China

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ABSTRACT

The phenolic profiles and antioxidant activity of litchi pulp of 13 varieties were investigated. The free, bound and total phenolic contents were 66.17–226.03, 11.18–40.54, and 101.51–259.18 mg of gallic acid equivalents/100 g, respectively. The free, bound and total flavonoid contents were 16.68–110.33, 10.48–22.75, and 39.43–129.86 mg of catechin equivalents/100 g, respectively. Free phenolics and flavonoids contributed averagely 80.1% and 75% to their total contents, respectively. Six individual phenolics (gallic acid, chlorogenic acid, (+)-catechin, caffeic acid, (−)-epicatechin, and rutin) were detected in litchi pulp by HPLC. The contents of each compound in free and bound fractions were determined. Significant varietal discrepancy in antioxidant activity was also found by FRAP and DPPH scavenging capacity methods. Antioxidant activity was significantly correlated with phenolic and flavonoid contents. Thus, phenolics and flavonoids exist mainly in the free form in litchi pulp. There were significant varietal differences in phytochemical contents and antioxidant activity of litchi pulp.

1. Introduction

Many chronic diseases, such as cardiovascular diseases and cancer, are the leading causes of death in many developed and developing countries. Epidemiologic evidence has suggested that diets rich in fruits and vegetables are associated with reduced risk of these diseases. Phytochemicals in fruits and vegetables are known to be associated with many health benefits. One could be that the potent antioxidant properties of phytochemicals may decrease oxidative stress in consumers. Previous research showed that phenolic compounds contribute most to the antioxidant capacity of fruits and vegetables (Eberhardt, Lee, & Liu, 2000).

Litchi (*Litchi chinensis* Sonn.), which originates in China, is now cultivated widely in warm climates around the world. It becomes one of the world's most popular fruits due to its delicious flavor and attractive appearance. As good sources of minerals, dietary fibre, and phytochemicals (Mahattanatawee et al., 2006), litchi has been employed in traditional Chinese medicine for its benefits on heart, spleen and liver. However, little is known about the possible bioactive compounds contributing to the health benefits of litchi.

Previous studies on the phenolic content and antioxidant activity of litchi fruit were mainly focused on its by-products, pericarp (Sarni-Manchado, Le Roux, Le Guerneve, Lozano, & Cheynier, 2000) and seeds (Nagendra Prasad et al., 2009). In contrast, the most commonly consumed part, litchi pulp, was rarely mentioned in the literature. Both qualitative and quantitative knowledge of phenolics and their antioxidant activity in the pulp are limited and incomplete. Amitabye et al. investigated the total phenolic content and antioxidant activity of litchi pulp in northern Mauritius (Luximon-Ramma, Bahorun1, & Crozier, 2003). Mahattanatawee et al. (2006) detected glycosides of quercetin and kaempferol in litchi pulp from southern Florida. However, these studies only reported the free phenolics on the basis of the solvent-soluble extraction, while the bound phenolics and their contribution to antioxidant activity were not involved. In fact, it was reported that approximately 24% of total phenolics in fruits were presented in the bound form (Sun, Chu, Wu, & Liu, 2002). As indicated in previous studies (Chandrasekara & Shahidi, 2010; Madhujith & Shahidi, 2009), there will be an underestimation of the phenolic content and antioxidant activity if the bound fraction was not included. Furthermore, many litchi cultivars mature in different time periods. Their phenolic compositions and activities are

Abbreviations: DPPH, 2,2-diphenyl-1-picrylhydrazyl; FRAP, ferric reducing antioxidant power; GAE, gallic acid equivalents; CE, (+)-catechin equivalents; TE, trolox equivalents.

* Corresponding author at: Bio-Technical Research Institute, Guangdong Academy of Agricultural Sciences, Guangzhou 510610, PR China. Tel.: +86 20 87237865; fax: +86 20 87236354.
E-mail address: mwzhh@vip.tom.com (M. Zhang).

[1] These authors contributed equally to this work.

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assumed to differ due to the differences in cultivation environment and varieties. However, only a certain litchi cultivar was analysed in previous research. It will greatly enhance our knowledge in terms of the nutritive values of these diverse cultivars if the differences in phenolic distribution and antioxidant activity among them are available.

Therefore, 13 representative litchi cultivars in southern China were analysed in the present investigation to determine (1) the contents of free and bound phenolics and their antioxidant activities; (2) the compositions and contents of individual phenolics in both free and bound forms; (3) the correlation among phenolic contents and antioxidant activities.

2. Materials and methods

2.1. Chemicals and reagents

Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), DPPH (2,2-diphenyl-1-picrylhydrazyl), TPTZ (2,4,6-tripyridyl-*s*-triazine), gallic acid, chlorogenic acid, (+)-catechin, caffeic acid, (−)-epicatechin, rutin and Folin–Ciocalteu's phenol reagent were purchased from Sigma Chemical Co. HPLC-grade acetic acid and acetonitrile were obtained from Fisher (Suwanee, GA, USA). All other chemicals used were of analytical grade or above.

2.2. Litchi fruit preparation

Different Litchi cultivars at commercial maturation season were obtained from the local markets from April to July 2011. Mature litchi fruits that were free from visible blemish or disease were selected. Fresh fruits were peeled soon after harvest and the pulp portion was kept in sealed polyethylene bags at −40 °C for further analysis.

2.3. Extraction of free phenolics

Free phenolics were extracted according to the previous methods (Sun et al., 2002). Briefly, 100 g of fresh litchi pulp was homogenised with chilled 80% aqueous acetone (1:2, w/v) in a Philips blender for 5 min. The sample was then further homogenised using an XHF-D homogenizer at 5000 rpm for an additional 3 min at 4 °C (Ningbo Xin-zhi-Bio Technology Co. Ltd., Ningbo, China). The homogenates were filtered through No. 2 Whatman filter paper on a Buchner funnel under vacuum. The residue was extracted again under the same conditions. The filtrates were combined and concentrated under vacuum at 45 °C until approximately 90% of the filtrate had been evaporated. The concentrated filtrate was then recovered with distilled water to a final volume of 50 mL and then stored at −40 °C until use.

2.4. Extraction of bound phenolics

Bound phenolics were extracted according to the previous literature (Naczk & Shahidi, 1989; Sun et al., 2002). Briefly, the residue from the above free phenolics extraction was hydrolyzed with 20 mL of 4 M NaOH at room temperature for 1 h with continuous shaking under nitrogen gas. The mixture was then neutralised with concentrated hydrochloric acid and extracted six times with ethyl acetate. The ethyl acetate fractions were combined and evaporated at 45 °C to dry. The bound phenolics were reconstituted with distilled water to a final volume of 10 mL and then stored at −40 °C until use.

2.5. Determination of total phenolic contents

The contents of total phenolics were analysed by the Folin–Ciocalteu (FC) colorimetric method described previously by Dewanto, Wu, Adom, and Liu (2002). Briefly, a 125 μL of the above extract was mixed with 0.5 mL of distilled water and subsequently with 125 μL of FC reagent. After 6 min, 1.25 mL of 7% aqueous sodium carbonate solution was added into the mixture. Then water was added to bring the total volume to 3 mL. The colour developed for 90 min, and the absorbance was measured at 760 nm using a Shimadzu UV-1800 spectrometer (Shimadzu Inc., Kyoto, Japan). Gallic acid was used as the standard, and total phenolic content was expressed as milligrams of gallic acid equivalents (GAE) per 100 g of fresh weight (FW) of sample.

2.6. Determination of total flavonoids contents

The total flavonoids contents of the litchi extracts were determined using a modified colorimetric method described previously (Dewanto et al., 2002). A 250 μL aliquot of the above extract was added to a tube containing 1.25 mL of distilled water. To the mixture was added subsequently 75 μL of 5% $NaNO_2$ solution. After 6 min, 150 μL of 10% $AlCl_3{\cdot}6H_2O$ solution was added and allowed to stand for 5 min before further addition of 0.5 mL of 1 M NaOH solution. Distilled water was added to bring the total volume to 2.5 mL, and the absorbance was read immediately at 510 nm using a Shimadzu UV-1800 spectrometer. Total flavonoids contents were expressed as milligrams of (+)-catechin equivalents (CE) per 100 g of FW of sample. Additional dilution was needed if the absorbance measured was over the linear range of the (+)-catechin standard curve.

2.7. Determination of phenolic compositions

All the samples were analysed on an Agilent 1200HPLC system (Waldbronn, Germany) equipped with an Agilent 1200 series VWD detector and autosampler, using a 250 × 4.6 mm id, 5 μm Agilent Zorbox SB-C_{18} column (Palo Alto, CA, USA). Column temperature was maintained at 30 °C. The mobile phase consisted of 0.4% aqueous solution of acetic acid (solution A) and acetonitrile (solution B). The gradient was programmed as follows: 0–40 min, solution B 5–25%; 40–45 min, solution B 25–35%; 45–50 min, solution B 35–50%. Other chromatographic conditions included a constant flow rate of 1.0 mL/min, an injection volume of 20 μL, and a run time of 50 min. Detection was set at 280 nm. Prior to analysis, all of the samples were filtered through a 0.25 μm membrane filter (Millipore, Billerica, MA, USA). Identification of each peak was primarily based on comparison of their retention times with the known authentic standards. The percent recovery of these phenolics was from 85.0% to 105.3%.

2.8. Antioxidant activity determined by FRAP assay

The FRAP assay was carried out according to a modified previous method (Benzie & Strain, 1996). The working solution was prepared by mixing 25 mL of 300 mM acetate buffer (3.1 g of $CH_3COONa{\cdot}3H_2O$ and 16 mL of CH_3COOH pH 3.6), 2.5 mL TPTZ solution (10 mM TPTZ in 40 mM HCl), and 2.5 mL of 20 mM $FeCl_3{\cdot}6H_2O$ solution and incubating at 37 °C before use. Litchi pulp extracts as samples or distilled water as blank (200 μL) were allowed to react with 2.8 mL of the working solution for 30 min in dark at room temperature. Absorbance was determined at 593 nm using a Shimadzu UV-1800 spectrometer. Trolox was used as standard to establish a standard curve. The FRAP antioxidant activity was expressed as milligrams of trolox equivalents (TE) per 100 g of FW of sample.