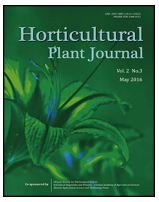
May 2016. Horticultural Plant Journal, 2 (3): 133–140.

CSHS

CrossMark

Horticultural Plant Journal

Available online at www.sciencedirect.com
The journal's homepage: http://www.journals.elsevier.com/horticultural-plant-journal



Phenolic Composition and Antioxidant Capacities of Chinese Local Pummelo Cultivars' Peel

LÜ Zefang [a], ZHANG Zhao [a], WU Hongmei [a], ZHOU Zhiqin [a,b], and YU Jie [a,b,*]

[a] *College of Horticulture and Landscape Architecture, Southwest University, Chongqing 400716, China*
[b] *Key Laboratory of Horticulture Science for Southern Mountainous Regions, Ministry of Education, Chongqing 400715, China*

Received 22 February 2016; Received in revised form 4 April 2016; Accepted 1 May 2016

Available online 24 August 2016

Abstract

China is one of the main production areas of pummelo [*Citrus grandis* (L.) Osbeck.] in the world and has lots of distinctive local cultivars. Systematic research on the detection of phenolics and antioxidant capacity of peels of mature local cultivars pummelo fruits is rare. In the current study, phenolic composition and content in peels (flavedo and albedo) of ten Chinese local pummelo cultivars were determined using Ultraviolet Spectrophotometer and High Performance Liquid Chromatography (HPLC), and their antioxidant capacities were evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals, ferric reducing antioxidant power (FRAP), and 2,2′-azino-bis (3-ethylbenzthiazoline-6)-sulfonic acid (ABTS) methods in the current study. The research not only provides data that support making full use of the resources of Chinese local pummelo cultivars, but also lays the theoretical basis for research of pummelo fruit nutrition and health values. The results showed that the total phenolic (TP) content in albedo was significantly higher than that in flavedo; on the contrary, the total flavonoid (TF) content was lower in the albedo than in the flavedo. *C. grandis* 'Hongxinyou' flavedo contained the highest TP contents, *C. grandis* 'Liangpingyou 78-8' flavedo contained the highest TF contents, and *C. grandis* 'Guanxi Miyou' albedo contained the highest TP and TF contents. Naringenin, hesperetin, diosmin, and gallic acid were the predominant phenolics in the flavedo, whereas hesperetin, diosmin, rutin, chlorogenic acid, and gallic acid were the primary phenolics in the albedo. The flavedo of *C. grandis* 'Yubei Shatianyou' and the albedo of *C. grandis* 'Dianjiang Baiyou' had the highest antioxidant potency composite (APC) indexes. Overall, the 'Yubei Shatianyou' flavedo and the 'Dianjiang Baiyou' albedo are excellent sources of antioxidants and have the greatest potential value for exploitation and utilization.

Keywords: pummelo; phenolics; flavonoid; phenolic acid; antioxidant capacity

1. Introduction

Polyphenols are one class of secondary metabolites in plants. They not only play an important role in plant resistance against pests and diseases, but also are important dietary nutritional sources for human beings. As one of the most important fruits in the world, citrus is a large family including sweet orange (*Citrus sinensis*), mandarin or tangerine orange (*C. reticulata*), grapefruit (*C. paradisi*), lemon (*C. limon*), and lime (*C. aurantifolia*). Pummelo is a major type of cultivated citrus species. Their fruit not only has a unique flavor, but also high nutritional value. Previous studies have proved that pummelo's health-promoting properties mainly depend on flavonoids and phenolic acids, which have anti-inflammatory and anti-cancer characteristics, that may play a role in the prevention of cardiovascular disease, diabetes, and other diseases (Viuda-Martos et al., 2010; Zhang et al., 2014).

China is one of the major production areas of pummelo and has long cultivation history for this crop. It has been found that there are more than 120 pummelo cultivars (Zhang et al., 2001). According to geography, climate, and other natural factors, pummelo has three producing centers in China, namely, the South China district, the Southeast coastal region, and the Southwest and South regions (Ran et al., 2013). In recent years, many researches on pummelos have been focused on field genetics using molecular markers to investigate genetic mapping (Chai et al., 2013), identify germplasm resources (Chen et al., 2012), etc. With the exploration of bioactive substance compounds and the

* Corresponding author. Tel.: +86 23 68250229.
E-mail address: yujie1978@126.com

Peer review under responsibility of Chinese Society for Horticultural Science (CSHS) and Institute of Vegetables and Flowers (IVF), Chinese Academy of Agricultural Sciences (CAAS)

http://dx.doi.org/10.1016/j.hpj.2016.05.001

antioxidant capacity of citrus fruits such as sweet orange, lemon (Abad-García et al., 2012a), grapefruit (Goulas and Manganaris, 2012), orange (Chen et al., 2010), and mandarin (Zhang et al., 2014), researchers are currently studying the phenolic composition (He et al., 2011; Goulas and Manganaris, 2012) and antioxidant capacity in each part of the pummelo (Fang et al., 2013; Xi et al., 2014). However, systematic studies on the detection of active substances and antioxidant capacity of the flavedo and albedo of mature fruits of Chinese local pummelo cultivars are rare.

The objective of the present study is to characterize the phenolic content and composition of local Chinese pummelo flavedos and albedos, and to evaluate the total antioxidant capacities of the flavedos and albedos. This research will provide the information for pummelo plant cultivation and the protection and utilization of Chinese pummelo resources.

2. Materials and methods

2.1. Plant material

All local citrus cultivars which are well-known in China and the main varieties of Chongqing were collected from the main pummelo production areas in Chongqing, China (Table 1). Fruits were harvested at the commercial maturity stage (late November) of eight-year-old seedlings based on external color and size uniformity. After harvest, peel samples of each cultivar were divided into flavedo and albedo, cut into small pieces, dried to a moisture content of less than 5%, and sieved through 60-mesh sieve. Then the powders were stored in the dryer for until use.

2.2. Chemicals

Eriocitrin, hesperidin, rutin, diosmin, hesperetin, sinensetin, nobiletin, tangeretin, gallic acid, chlorogenic acid, and ferulic acid were purchased from J & K Scientific Ltd. (Beijing, China). Eriodictyol, naringenin, caffeic acid, methanol, formic acid, trolox, DPPH, 2,4,6-tripyridyl-s-triazine (TPTZ), and ABTS were all obtained from Sigma (St. Louis, MO, USA). Other analytical reagents were obtained from Chengdu Kelong Chemical Reagent Co. (Chengdu, China).

Table 1 The pummelo cultivars used in the present study

No.	Cultivar	Abbreviation	Origin
1	Wubuyou	WB	Banan, Chongqing, China
2	Changshou Shatianyou	CS	Changshou, Chongqing, China
3	Dianjiang Baiyou	DJ	Dianjiang, Chongqing, China
4	Hongxinyou	HX	Fengdu, Chongqing, China
5	Kuiyou	KY	Fengjie, Chongqing, China
6	Guanxi Miyou	GX	Hechuan, Chongqing, China
7	Humiyou 1	HM	Liangping, Chongqing, China
8	Liangpingyou 78-8	LP	Liangping, Chongqing, China
9	Yubei Shatianyou	YB	Yubei, Chongqing, China
10	Zhenlongyou 3	ZL	Zhongxian, Chongqing, China

2.3. Phenolics extraction

Eight milliliters of methanol was added to 0.4 g of fruit sample. The mixture was shaken completely, extracted using ultrasonic ions for 30 min at 50 °C, and then centrifuged at 5 000 × *g* for 15 min. Supernatants were extracted twice with the same procedure. All supernatants were merged and diluted to 25 mL with methanol. The solutions were then stored at −20 °C (Ramful et al., 2011; Ran et al., 2013).

2.4. Determination of TP and TF

TP was determined using the Folin–Ciocalteu method with some modifications (Singleton et al., 1999); 0.25 mL extract, 0.75 mL ddH_2O, and 1 mL Folin–Ciocalteu were mixed in 10 mL centrifuge tubes and shaken thoroughly. After standing for 5 min without light, 1 mL of 5% Na_2CO_3 solution was added and mixed. After 60 min, the absorbance was detected at a wavelength of 765 nm using ultraviolet spectrophotometry.

TF content was determined by the method of Kim et al. (2003) with some modifications; 0.7 mL ddH_2O was added to 0.5 mL extracts and mixed completely. Then 0.3 mL 5% $NaNO_2$ was added and the mixture was kept for 5 min. After adding 0.2 mL 10% $Al(NO_3)_3$, the mixture was incubated for 6 min, and then 2 mL 1 mol · L^{-1} NaOH was added. After incubating for 15 min at room temperature, the absorbance was detected at a wavelength of 500 nm.

2.5. Phenolics determination by HPLC

Phenolic standards (10.00 mg each) were dissolved in methanol and diluted to 10 mL. The phenolic compounds in the flavedo and albedo extracts of each genotype were detected according to HPLC methods (Zhang et al., 2012). After filtration through a Millipore membrane (0.22 μm), 10 μL of solution was injected into the HPLC system. Chromatographic separation was performed using a reverse phase column (Diamonsil C18, 250 mm × 4.6 mm internal diameter). The mobile phase was composed of methanol A and aqueous B at a flow rate of 0.7 mL · min^{-1}. The column temperature was maintained at 25 °C. Gradient elution was performed as described in Table 2. The five flavanone standards were detected at a wavelength of 283 nm. The four flavone standards were detected at a wavelength of 330 nm. Rutin was the only flavonol standard used in this study that was detected at a wavelength of 367 nm. Four phenolic acid standards were detected at wavelengths of 260 nm and 320 nm. The liquid chromatograms of all samples were

Table 2 Program of gradient elution

Time/min	Methanol/%	0.1% formic acid (aqueous)/%
0	37	63
20	50	50
35	80	20
40	100	0
50	100	0
60	37	63