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Chemical features of *Pericarpium Citri Reticulatae* and *Pericarpium Citri Reticulatae Viride* revealed by GC–MS metabolomics analysis

Lunzhao Yi [a,*], Naiping Dong [b], Shao Liu [e], Zhibiao Yi [c], Yi Zhang [d,*]

[a] *Yunnan Food Safety Research Institute, Kunming University of Science and Technology, Kunming 650500, China*
[b] *Department of Applied Biology and Chemical Technology, The Hong Kong Polytechnic University, Hong Kong 999077, China*
[c] *Dongguan Mathematical and Engineering Academy of Chinese Medicine, GuangZhou University of Chinese Medicine, Dongguan 523808, China*
[d] *College of Chemistry and Chemical Engineering, Central South University, Changsha 410083, China*
[e] *Xiangya Hospital, Central South University, Changsha 410008, China*

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Chemical compounds studied in this article:
D-limonene (PubChem CID: 440917)
Linalool (PubChem CID: 6549)
4-Carene (PubChem CID: 530422)
3-Carene (PubChem CID: 26049)
β-Pinene (PubChem CID: 14896)
γ-Terpinene (PubChem CID: 7461)
Geranyl acetate (PubChem CID: 1549026)
Farnesyl acetate (PubChem CID: 94403)
6-Hepten-1-ol (PubChem CID: 543123)
3-Methyl-1-hexanol (PubChem CID: 25793)

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Flavour
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ABSTRACT

This paper introduces a detailed method to apply metabolic profiles conducting on tangerine peels (*Citrus reticulata 'Dahongpao'*) at three maturity stages from July to December. Principal component analysis not only demonstrated the metabolic footprints of tangerine peels during ripening but also revealed the compounds (D-limonene and linalool) that mostly contributed to it. Furthermore, some other characteristic compounds were screened to further reveal the chemical features of *Pericarpium Citri Reticulatae* (PCR) and *Pericarpium Citri Reticulatae Viride* (PCRV). In particular, compounds such as 4-carene ($r = -0.94$), 3-carene ($r = -0.91$), β-pinene ($r = -0.85$) and γ-terpinene ($r = -0.87$) were screened as major components for the pungent smell of PCRV. Geranyl acetate ($r = 0.81$), farnesyl acetate ($r = 0.87$) and three alcohols (6-hepten-1-ol, 3-methyl-1-hexanol, 1-octanol) may lead to the pleasant odour of PCR. We therefore propose that the metabolomics analysis focusing on ripening process will be an effective strategy for quality control of closely related herbal medicines.

1. Introduction

Tangerine is one of the most popular fruits. The flesh could be eaten directly or be juice. The peels could be used as food or herbs. *Pericarpium Citri Reticulatae* (PCR) and *Pericarpium Citri Reticulatae Viride* (PCRV) are both from tangerine peels (Liu et al., 2013; Zheng et al, 2013). There are many closely related herbs, such as PCR and PCRV, in traditional Chinese medicines (TCMs), whose plant sources are the same while harvest times are different. Although their plant sources are the same, their chemical compositions should be different, leading to their different clinic applications. *Chinese Pharmacopoeia* describes like that the dried pericarps of the ripe fruit of *Citrus reticulate Blanco* or its cultivars collected from September to December are used for PCR, while peels collected from July to August are PCRV (Committee, 2010). Harvest time is determined just according to traditional experiences without scientific research validation. Their respective chemical features are unclear until now. The recently developed metabolomics analyses are very effective to clearly reveal changing trend of metabolites during ripening (Besada, Salvador, Sdiri, Gil, & Granell, 2013; Falasca et al., 2014; Toffali et al., 2011). They provide us an opportunity to reveal the different chemical features of the closely related TCMs.

In the past, only the evolving of the physiological parameters in tangerine fruits during ripening attracts attentions, including changes in colour, size, weight, acidity and flavour. According to previous studies, external colour can be used as a non-destructive maturity index for tangerine harvests, and the green one is for PCRV, the orange one is for PCR, although their chemical differences are unknown. More importantly, flavour is an essential index to evaluate the quality of PCRV and PCR according to traditional

* Corresponding authors. Tel.: +86 871 65920302 (L. Yi), +86 731 88836954 (Y. Zhang).
E-mail addresses: ylz7910@hotmail.com (L. Yi), yzhangcsu@csu.edu.cn (Y. Zhang).

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experience. The good PCR should be smelled pleasant, pure and mild. In this study, the associations between the volatiles and aroma/flavour of tangerine peels are discussed to reveal their chemical features corresponding to the special odour.

Some literatures have reported that metabolites in essential oils are main active compounds of PCR and PCRV, which have strong pharmacologic bioactivities (Blanco Tirado, Stashenko, Combariza, & Martinez, 1995; Flamini, Tebano, & Cioni, 2007). For example, D-limonene, as one of the bioactive components in PCRV and PCR volatile oils, showing the ability to the increase the expectoration rate and possess anticancer activities (Asamoto et al., 2002; Bezerra, Costa, & Nogueira, 2013; Shen, 2002). Significant antimicrobial activity of α-terpineol has also been proved (Carson & Riley, 1995; Cosentino et al., 1999). It also has been demonstrated that 4-terpineol owned the bacteriostatic activity against several micro-organisms (Barel, Segal, & Yashphe, 1991). However, so far, only a limited number of studies have addressed the identification of the volatile profiles of PCR or PCRV (Fan et al., 2000; Tu, Thanh, Une, Ukeda, & Sawamura, 2002), few of them involved the main associations between volatiles and ripening traits of tangerine peels (Wang & Liu, 2014).

In this study, we collected twenty-four samples of tangerine peels (*Citrus reticulata 'Dahongpao'*) at three maturity stages, covering the first fruit bearing and complete ripening, from July to December. Our studies were focused on the alterations of volatile metabolites of tangerine peels from different maturity stages, revealing the chemical features of PCR and PCRV. Firstly, GC–MS technique was employed to generate the informative metabolic profiles of samples under the optimum analytical conditions. Next, metabolites were identified by mass and temperature-programmed retention indices (PTRIs), and semi-quantified by relative peak areas. After that, principal component analysis (PCA) was applied to investigate the metabolic footprints of tangerine peels and screen the characteristic components which have relative higher contributions. Finally, a heat map and Pearson correlation analysis were employed to screen the characteristic compounds representing the three different ripening stages. In addition, changing trend of every marked component was analysed in details, respectively.

2. Materials and methods

2.1. Materials

The *Citrus Reticulata 'Dahongpao'* (of Zigong city, Sichuan province) cultivar was chosen for its good quality, according to traditional experiences. Four tangerine trees in an orchard and on the sunny side were selected. Tangerine fruits during the whole maturity period (from July to December) were monthly harvested from the original cultivation place. Fruits were carefully collected on each harvest day from individual tree, respectively. At each maturation/ripening stage, homogenous lots of fruits each were made up (four replicates per maturity stage), colour and smell were recorded (Supplementary Table S1).

After the fruits had been washed, they were cut into four equal portions and the flesh was removed. Then, the peels of those tangerines were disposed according to the criterion in *Chinese Pharmacopoeia* (Committee, 2010). They were cut into pieces and dried in sunless condition after impurities discarded. After that, twenty-four samples were obtained including PCRV and PCR. The photos of tangerine peels were shown in Supplementary Fig. S2. All samples were authenticated by Prof. Pei-shan Xie from Zhuhai Kingman Institute of Herbal Medicine Research, Guangdong province, China. The specimens are preserved in that institute now.

2.2. Extraction of volatile oil

Firstly, all crude samples were dried for about 60 min at 40 °C and smashed. Subsequently, 30 g of sample was swollen with

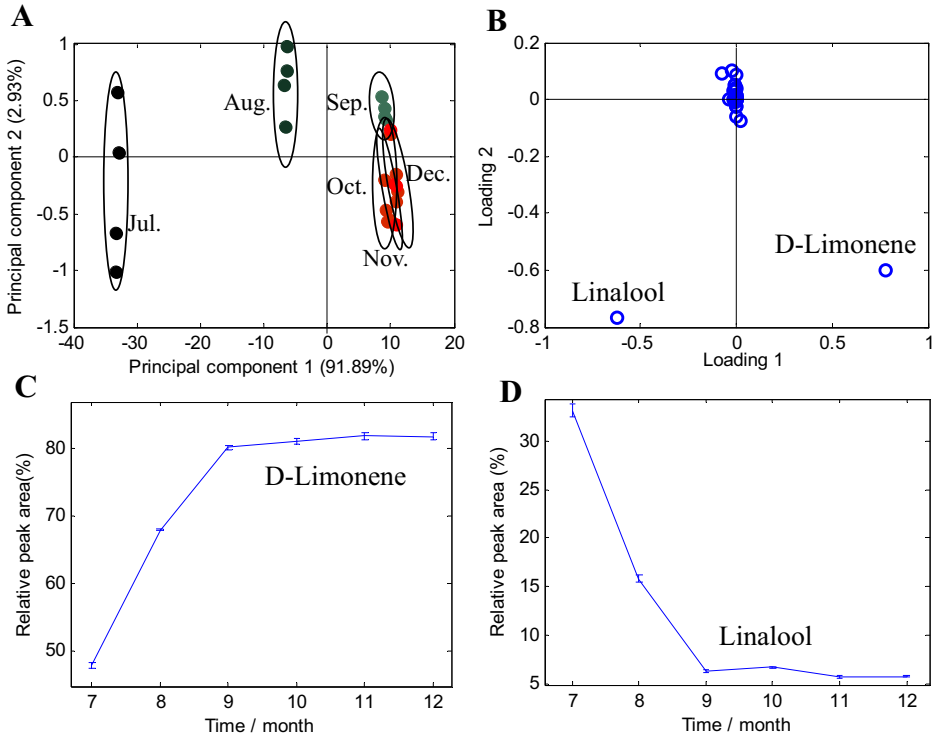

Fig. 1. A principal component analysis based on the volatile metabolic profiling results of tangerine peels at the total ripening stages. The size of matrix is 24 × 82. Scores and loadings' plots for the first and the second principal components are shown. Four replicates were analysed per maturity stage (from July to December). Linalool and D-limonene were identified by the loadings' plot. Their contents have different changing trends during the ripening process.

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