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Effects of cross-pollination by 'Murcott' tangor on the physicochemical properties, bioactive compounds and antioxidant capacities of 'Qicheng 52' navel orange

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ABSTRACT

This study investigated the effects of cross-pollination by 'Murcott' tangor on the fruit quality of 'Qicheng52' navel orange, including the physicochemical properties, bioactive compounds and antioxidant capacities. There were no significant differences on the fruit weight, juice yield and pH value of juice between self- and cross-pollinated fruits. However, cross-pollination could significantly improve the fruit quality of 'Qicheng52' fruits by increasing the total soluble solid content from 11.12 ± 1.02 °Brix to 13.86 ± 1.17 °Brix. The results of high performance liquid chromatography analysis of three sugar components indicated that the increase of total sugar was mainly contributed by the increase of fructose and sucrose. Cross-pollination exhibited no effect on the flavonoids content, while the total phenolics content was increased from 210.09 ± 18.55 mg/L to 298.25 ± 29.10 mg/L, which contributed to the higher antioxidant capacity in the cross-pollination fruit juice.

1. Introduction

Citrus is an important crop worldwide, and the delicious flavor and beneficial health effects of citrus fruits are the important factors for their market values. Sugars and organic acids are the major components in citrus fruit juice, and their compositions and concentration largely affect taste characteristic and organoleptic quality (Liu, Yang, & Deng, 2015). Citrus fruits have been well accepted as an dietary source of bioactive compounds, such as ascorbic acid, phenolic compounds and flavonoids, which are found to be abundant in citrus fruits and associated with reduced risk of certain chronic diseases (Xi et al., 2014). In recent years, antioxidant properties of citrus fruits are becoming a major factor in determining consumer acceptance and preference (Loizzo et al., 2018; Zou, Xi, Hu, Nie, & Zhou, 2016). A large amount of evidence indicates that the antioxidant capacity of citrus fruits strongly correlate with bioactive compounds, such as phenolic compounds (Lagha-Benamrouche, & Madani, 2013; Legua, Forner, Hernández, & Forner-Giner, 2014; Xi et al., 2014).

Currently, there are increasing interests in the fruit quality of citrus species, which appeared to be affected by farming methods and rootstocks combinations (Legua, Bellver, Forner, & Fornerginer, 2011; Legua et al., 2014; Letaief, Zemni, Mliki, & Chebil, 2016). Cross-pollination has been used as an effective method to increase the yield or quality of crops (Fattahi, Mohammadzedeh, & Khadivi-Khub, 2014; Shemer et al., 2014; Sulewska et al., 2014). In the recent literatures, cross-pollination between citrus species was proved to be an effective method to increase the yield of the citrus cultivars suffering from inadequate yield (Schneider, Goldway, Rotman, Adato, & Stern, 2009). However, the effects of cross-pollination on the citrus fruit quality, including weight, total soluble solid (TSS) content, total acids (TA), juice content and rind thickness, have not been comprehensively investigated in the previous research (Papadakis, Protopapadakis, & Therios, 2009). In our recent studies, 'Murcott' tangor (*Citrus reticulata* Blanco × *Citrus sinensis* Osbeck) trees, using as a pollenizer, were mix-planted with 'Qicheng 52' navel orange (*Citrus sinensis* Osbeck) trees. The fruit quality of 'Qicheng 52' could be significantly increased, especially for its TSS content.

'Qicheng 52' navel orange, developed from the fine mutation lines of 'Newhall' navel orange (*Citrus sinensis* Osbeck), is a new high-quality navel orange cultivar, which has become one of the main citrus varieties in southern China. The oval and seedless 'Qicheng 52' fruits have the TSS content ranged from 11.5% to 13.0% and the TA ranged from

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0.46% to 0.73% (Zhao, Guo, Chen, Yu, & Xu, 2010). 'Qicheng 52' trees are always cultivated in isolated blocks, therefore little information is available about the effects of cross-pollination on its yield or fruit quality. By accident, we found that 'Murcott' tangor, a sweet orange with high TSS content, could be used as a potential pollenizer for 'Qicheng 52'. The cross-pollination might not increase its yield, but could be an efficient and cheap method to increase the fruit quality of 'Qicheng 52', and thus significantly increase its market value.

In current study, the organic acids, sugars, phenolic composition and antioxidant capacity of the orange juice from the 'Qicheng 52' have been determined. The effect of cross-pollination by 'Murcott' tangor on the 'Qicheng 52' fruit qualities was investigated, focusing on the contents of bioactive compounds and the antioxidant capacities.

2. Material and methods

2.1. Chemicals

HPLC grade sugars and organic acids standards, Folin–Ciocalteureagent, trolox (6-hydrox-2,5,7,8-tetramethylchromane-2-carboxylic acid), AAPH (2,2'-azobis (2-methylpropionamidine) dihydrochloride), TPTZ (2,4,6-tris(2-pyridyl)-s-triazine), galic acid and catechin were purchased from Sigma (St. Louis, MO, USA). All other chemicals used in this study were of analytical grade.

2.2. Plant materials and sample preparation

The experiments were conducted in two different commercial 'Qicheng 52' navel orange orchards in Fujian province, China. One orchard was used as 'self-pollination (SP)' orchard, which consisted of 'Qicheng 52' trees with at least 2 km distance from the nearest 'Murcott' tangor trees or any other citrus varieties. Another orchard was used as 'cross-pollination (CP)' orchard, which has three rows of 'Murcott' tangor trees among the rows of 'Qicheng 52' trees (Fig. 1). The two orchards have similar soil type, growing conditions and farming methods.

Thirty homogeneous 'Qicheng 52' fruit samples were randomly chosen from trees in the SP orchard, and another thirty samples from the 'Qicheng 52' trees adjacent to 'Murcott' tangor trees in the CP orchard for the experiments. Thirty fruit samples were separated into three groups and weighted, then the edible fresh was collected by manual peeling, mixed and carefully extracted in a commercial kitchen juicer. For the physicochemical analyses, the resulting fruit juice was centrifuged at 5000*g* for 20 min at 4 °C, and the supernatant was collected and kept at −18 °C until analysis. For determination of total phenolics, total flavonoids and antioxidant capacity, 1 mL of fruit juice was extracted with 9 mL of 80% v/v methanol for 30 min at room temperature. After centrifugation at 5000*g* for 10 min, the supernatant was recovered and kept at -18 °C until analysis.

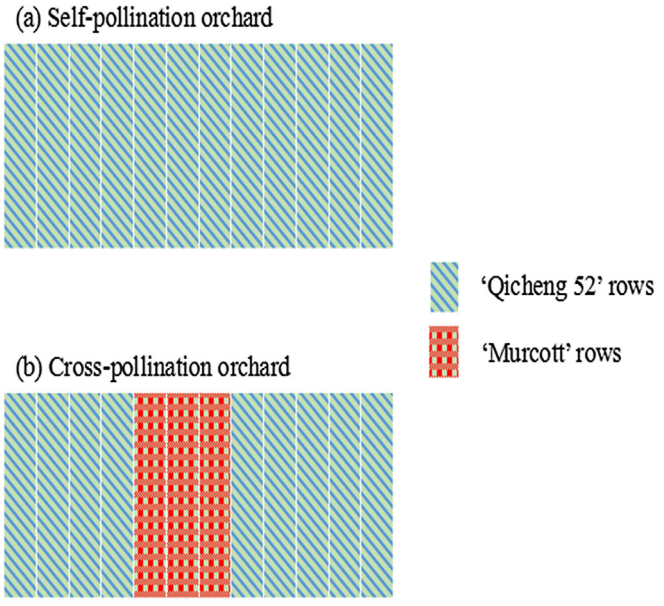

Fig. 1. Schematic diagram of self- and cross-pollination orchards of 'Qicheng 52' navel orange. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2.3. Physicochemical properties

The fruit weight was recorded, and the juice yield was calculated after juicing. The physicochemical properties of juice samples, i.e. pH, TA and TSS, were determined in triplicate. The pH value was measured using a pH meter (PHS-300, Shengaohua, China). TA was quantified by titrating 1 g of the supernatant with 0.1 M NaOH, and then expressed as g of citric acid/L of juice (Legua et al., 2014). The TSS content was determined in Brix with a hand-held refractometer (RX-5000a, Atago, Japan) and expressed as degrees Brix at 20 °C (Forner-Giner, Alcaide, Primo-Millo, & Forner, 2003). The ripening index (RI) was calculated as the relation between TSS and TA.

2.4. HPLC analysis of sugars and organic acids

To determine individual sugars and organic acids, the juice samples from SP and CP fruits (three replicates per treatment) were analyzed by high performance liquid chromatography (HPLC) method as described with some modifications (Cheong, Liu, Zhou, Curran, & Yu, 2012). Twenty microliters of extract was filtered through a 0.45 μm Millipore filter and then injected into a HPLC system (1260 Infinity, Agilent, USA). The sugar compounds were analyzed using Zorbax carbohydrate analysis column (250 mm × 4.6 mm, 5 mm, Agilent) at 40 °C, and the mobile phase was acetonitrile: water (70 : 30, v/v) with a flow rate of 0.7 mL/min. Sugars were detected with a refractive index detector.

To separate the organic acids, Supelco Supelcogel C-610H ion exchange column (300 mm × 7.8 mm) with sulfonated polystyrene divinylbenzene packing was selected, and the detection of the acids were measured at 214 nm using a UV/Vis detector. The mobile phase used was 0.10% H_2SO_4 with the flow rate of 0.40 mL/min. A standard curve of pure organic acids (oxalic, citric, malic, succinic and ascorbic acids) and a standard curve of pure sugars (glucose, fructose and sucrose) were used for quantification. Results for both individual organic acids and sugars were expressed as g per 100 g fresh weight.

2.5. Determination of total phenolic content

Based on the ability of the phenolic substances to form a blue molybdenum-tungstic complex with the reagent of Folin-Ciocalteu, the total phenolic content (three replicates per treatment) was determined as previously described with some modifications (Valchevakuzmanova et al., 2007). Briefly, 1 mL of methanolic extract was added to a 25 mL volumetric flask filled with 9 mL of distilled water. The mixture was added with 0.5 mL of Folin-Ciocalteu phenol reagent, and then mixed thoroughly. After standing for 60 min, the mixture was monitored at 760 nm using spectrophotometer (Unico UV-2000, Shanghai, China) versus the prepared blank. The total phenolic content was determined from standard curve of gallic acid (GA) and was expressed as mg of gallic acid equivalent (GAE, mg/L).

2.6. Determination of total flavonoids content

The total flavonoids content was measured by a colorimetric assay with minor modifications (Jia, Tang, & Wu, 1999; Letaief et al., 2016). Briefly, in a 10 mL volumetric flask, 250 μL of methanolic extract was sequentially added with 75 μL $NaNO_2$ (5% w/v), 150 μL $AlCl_3$ (10% w/