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Determination of sugars, organic acids, aroma components, and carotenoids in grapefruit pulps

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ABSTRACT

The composition and content of sugars, organic acids, volatiles and carotenoids, in the pulps of six grapefruit cultivars, were examined by HPLC and GC–MS. The results showed that sucrose was the dominant sugar in grapefruit, making up 40.08–59.68% of the total sugars, and the ratio of fructose to glucose was almost 1:1. Citric acid was the major organic acid and represented 39.10–63.55% of the total organic acids, followed by quininic acid. The ratios of individual sugars and organic acids play an important role in grapefruit taste determination. Monoterpenes and sesquiterpenes were the predominant volatiles in grapefruit, in particular D-limonene and caryophyllene. Caryophyllene, α-humulene, humulen-(v1), β-linalool and tert-butyl 2-methylpropanoate are the characteristic aroma compounds of grapefruit. Although β-carotene is the primary carotenoid in grapefruit, the pulp color is mainly determined by the ratios of zeaxanthin, β-cryptoxanthin and lycopene. Our results provide the first complete chemical characterization of the taste, aroma and color of grapefruit.

1. Introduction

Citrus is a large botanical family in which the dominant members are the sweet orange (*Citrus sinensis*), mandarin or tangerine orange (*Citrus reticulata*), grapefruit (*Citrus paradisi*), lemon (*Citrus limon*), and lime (*Citrus aurantifolia*). Grapefruit is one of the major commercial citrus crops, for both the fresh market and for processing (Chebrolu, Jayaprakasha, Jifon, & Patil, 2012). Grapefruits have a unique shape, flavor, color and a long shelf life, all qualities that are attractive to consumers. In addition, they are also an excellent source of many nutrients and phytochemicals that contribute to a healthy diet. Currently, there is an increasing interest in grapefruit because consumption appears to be associated with a reduced risk of certain chronic diseases, such as obesity, diabetes, cancers and cardiovascular disease (Kelebek, 2010).

Taste, aroma and color are important fruit quality factors that determine consumer preference. These traits also provide important information or sensory cues about the nutritional makeup of plant products (Goff & Klee, 2006; Kader, 2008). Grapefruit has a unique, special flavor and a colorful flesh. Its flavor is derived from a combination of its taste and aroma. The taste of grapefruit primarily depends on sugars and organic acids, whereas its aroma depends on a large number of volatile organic compounds (VOCs). Many studies have shown that the primary organic acids of citrus are citric and malic acid, and sucrose is present in large amounts in citrus fruit (Karadeniz, 2004). Previous studies have addressed how thermal treatment, storage (Igual, García-Martínez, Camacho, & Martínez-Navarrete, 2010) and hot air treatment influence the organic acid and sugar metabolism (Chen et al., 2012), sugar, organic acid, and phenolic composition of grapefruit (*C. paradisi* cvs. Rio Red, Star Ruby, Ruby Red and Henderson) (Kelebek, 2010), and the taste-related chemicals in *Ziziphus mauritiana* fruit (Muchuweti, Zenda, Ndhlala, & Kasiyamhuru, 2005). There is no doubt that volatile components play a determinant role in the grapefruit flavor quality. Many studies have also investigated the volatile components in pummelo peel (Cheong et al., 2011; Chung et al., 2012; Shao et al., 2014), essential oil (Sun et al., 2014) and juice (Cheong, Liu, Zhou, Curran, & Yu, 2012). Few researchers have investigated the volatile composition of grapefruit. Ren et al. (2015) characterized the free and bound volatile compounds from pink grapefruit and white grapefruit. Njoroge, Koaze, Karanja, and Sawamura (2005) analyzed the volatile constituents of Red Blush grapefruit (*C. paradisi*) peel essential oils from Kenya. The external color of citrus fruits is one of their most important quality traits, and it is a decisive factor for consumers.

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Grapefruit is characterized by its white, pink and red colors; the coloration of the pulp is primarily influenced by the presence of carotenoids (Rodrigo, Alquézar, Alós, Lado, & Zacarías, 2013). Alquezar, Rodrigo, Lado, and Zacarías (2013) analyzed the carotenoid biosynthetic differences between white and red grapefruit (*C. paradisi* Macf.). Xu, Fraser, Wang, and Bramley (2006) investigated the carotenoid content differences between ordinary citrus and mutant fruits. Alquezar et al. (2013) conducted a comparative physiological and transcriptional study of carotenoid biosynthesis in white and red grapefruit (*C. paradisi* Macf.). Despite previous studies, there is still much to be learned about grapefruit taste, aroma, and color composition.

The objectives of the current study were to identify the composition and content of soluble sugars, organic acids, volatile components and carotenoids in grapefruit pulps, and to create a comprehensive chemical characterization on the taste, aroma and color of grapefruit.

2. Materials and methods

2.1. Chemicals

Sugars (fructose, sorbitol, glucose and sucrose) and organic acids (oxalic acid, tartaric acid, quininic acid, malic acid, citric acid and aconitic acid) were all obtained from Shanghai Sangon Biological Reagent Company (Shanghai, China). *n*-Hexanol, methyl myristate, lutein, zeaxanthin, β-cryptoxanthin lycopene, α-carotene, and β-carotene were obtained from Sigma (St. Louis, MO, USA). All other reagents were of analytical grade and were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

2.2. Fruit materials

Six grapefruit (*C. paradisi* Macf.) cultivars were grown at the National Citrus Germplasm Repository in the Citrus Research Institute at the Chinese Academy of Agricultural Sciences, Chongqing, China (Table 1). All experimental trees were planted in 2001, in rows, in a north–south orientation, with a distance of 3–4 m between rows. Fertilization management and pest control were carried out according to standard practices of the germplasm repositories. During the 2014 harvest season (from the 12th to 30th of January), a total of 240 fruits were picked, from ten trees at the commercial maturity stage, on the basis of external color and size uniformity for each cultivar (Fig. 1). After harvest, fruits were randomly divided into three replicates and manually peeled. Only the pulp was used as the experimental material. Each replicate included 80 fruits. Among these fruits, 20 were used to determine the titratable acid (TA) and soluble solids content (SSC). Sixty grapefruits were ground into a fine powder in liquid nitrogen using a freezer-mill (6750) apparatus (Glen Creston), and then the powder was stored at -80 °C until analysis. In the study, three replicates were performed for all chemical analyses.

2.3. Soluble solids content and titratable acidity determination

SSC and TA were determined according to the method described by Ramful, Tarnus, Aruoma, Bourdon, and Bahorun (2011). Firstly, the pulp juice of peeled fruit was dropped on a digital refractometer (Atago PR-101R, Tokyo, Japan) and the value was read. Each replicate contained 20 fruits and all determinations were performed in triplicate. The temperature of the sample at the time of measurement was also recorded. The degree (°) Brix of the juice was then calculated and a temperature correction was applied. After measuring the SSC, the pulps of all 20 fruits were homogenized in a Waring blender and filtered with muslin cloth. Ten ml of the juice was diluted to 100 ml with distilled water and transferred into a 250 ml beaker, which was placed over a magnetic stirrer to provide continuous stirring of the sample solution. A pH meter probe was then immersed in the solution, and 0.1 N NaOH was added until the pH of the sample exceeded 8.1. TA was expressed as percentage of citric acid (%) and three replicates were used.

Table 1
Grapefruit cultivars used in the present study and their quality index values of pulps.[a,b,c,d]

No.	Repository number	Cultivars	Abbreviation	SSC (%)	TA (%)
1	LG0093	*C. paradis* cv. Marsh	MG	9.10 ± 0.01[d]	1.87 ± 0.09[b]
2	LG0120	*C. paradis* cv. Oroblanco	OR	11.53 ± 0.2[c]	0.90 ± 0.04[c]
3	LG0245	*C. paradis* cv. Cock Tail	CT	12.37 ± 0.31[b]	0.69 ± 0.03[d]
4	LG0094	*C. paradis* cv. Thompson	TG	11.77 ± 0.45[c]	1.92 ± 0.06[b]
5	LG0243	*C. paradis* cv. Red Blush	RB	13.27 ± 0.38[a]	2.14 ± 0.09[a]
6	LG0248	*C. parades* cv. Rio Red	RR	13.13 ± 1.02[a]	1.87 ± 0.03[b]

[a] SSC, soluble solid content.
[b] TA, titratable acidity.
[c] Data are expressed as means ± standard deviation of triplicate samples.
[d] Different lowercase letters between columns represent significant differences between cultivars ($p < 0.05$).

2.4. Determination of sugars and organic acids

Sugars and organic acids were extracted as described by Zhang et al. (2005). Two grams of pulp powder was homogenized by using 5.0 ml of cold ethanol (80%). The solution was then incubated for 20 min in a 35 °C water bath and centrifuged at 10,000×*g* for 10 min. This extraction procedure was repeated three times and the supernatants were combined. The total volume was then adjusted to 25 ml with 80% ethanol. From this mixture, 1 ml was dried under a vacuum (Eppendorf Concentrate Plus, Germany) at 45 °C, and the residue was resuspended in 0.5 ml of distilled water and filtered through a 0.22 μm, 13 mm diameter syringe filter (Shanghai Xingya Purification Material Factory, China). The filtered solution was then used for the sugar and organic acid analysis.

Sugars were analyzed as described previously with some modifications (Gancedo & Luh, 1986). A chromatographic separation of sugars involved acetonitrile: water (80:20, v/v) as the mobile phase at a flow rate of 1.4 ml/min with an Agilent ZORBAX Carbohydrate (4.5 μm, 4.6 mm × 250 mm) column (GL Sciences Inc., Torrance, CA, USA). Eluted peaks were detected with a SHODEX RI101 refractive index detector (JASCO International Co. Ltd, Tokyo, Japan). The data were analyzed with a Chromeleon® 6.8 chromatography data system (Thermo Fisher Scientific Inc., USA).

Organic acids were analyzed by HPLC, as described previously with some modifications (López-Hernández, Oruña-Concha, Simal-Lozano, Vázquez-Blanco, & González-Castro, 1996). The chromatographic separation used for organic acid detection employed $(NH_4)_2HPO_4$ (50 mM, pH 2.7) as the mobile phase, with a flow rate of 0.5 ml/min, and the samples were injected into an ODS C_{18} (4.6 mm × 250 mm) column (Beckman Coulter Inc., Brea, CA, USA). Organic acids were detected with a 2996 diode array detector (Waters Beckman Coulter Inc., Brea, CA, USA). The data were analyzed with a Waters Empower system.

Sugars and organic acids were detected at a wavelength of 210 nm. A calibration curve was prepared using commercial