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Sugars and organic acids changes in pericarp and endocarp tissues of pumelo fruit during postharvest storage

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ABSTRACT

Pumelo, a citrus fruit that originated from China and is widely planted there, is popular for its excellent quality and storage properties. Sugars and acids play critical roles in maintaining fruit quality. However, the detailed changes of sugars and acids that occur in pumelo are largely unknown. We measured the soluble sugars and organic acids concentrations in different tissues of Shatian pumelo (*Citrus grandis*), Guanxi pumelo (*C. grandis*) and Hirado Butun pumelo (HBP; *C. grandis* × *Citrus paradisi*) stored at ambient temperature (10–16 °C) by gas chromatography and high-performance capillary electrophoresis. During storage, organic acids and soluble sugars concentrations in the pulp fluctuated significantly instead of simply decreasing or increasing. The variations in citric, malic, aconitic and fumaric acid concentrations showed no substantial difference as did fluctuations in sucrose, fructose and glucose concentrations. In the pulp, the concentration of sucrose was a little higher than, or close to, that of glucose and fructose. However, the sucrose concentration in the pericarp was much lower than that of glucose and fructose. In the pulp of HBP, the change trend of sucrose concentration was opposite that of organic acid concentration after storage for 20 days post harvest (DPH). Sugar/acid ratios in HBP and Shatian pumelo increased with the storage time, while they decreased in Guanxi pumelo. The total concentration of soluble sugar decreased slightly in the pulp, but sucrose concentration decreased significantly (except in the Shatian pumelo); while in the pericarp, the total soluble sugar concentration showed a dramatic decreasing trend, indicating that carbohydrate transport from the pulp to pericarp or carbohydrate redistribution might occur in postharvest citrus fruit.

1. Introduction

Citrus fruits undergo a series of physiological and biochemical changes after harvest, which accelerate fruit aging and can produce flavor deterioration, color change, weight loss, softening, rotting and so on. Therefore, fruit storage methods have a significant impact on their shelf life. The ability to maintain fruit quality can be used as an important indicator to compare different storage methods, and is especially useful for sensory properties evaluation. Generally, the flavor of fruit is controlled by various types of chemical composition, and the balance between sugar and acid, which is crucial in controlling flavor changes in postharvest fruits. In stored 'Yali pear' (*Pyrus* × *bretschneideri* Reld) fruit, with the increase of respiration rate, the content of sugar and malic acid decreased steadily (Chen et al., 2006). In Japanese plums (*Prunus salicina* Lindl.) fruit, organic acids were depleted markedly as sugar declines during storage, resulting in significant changes of fruit flavor and shortened shelf life (Singh et al., 2009).

Sugar is the major component affecting fruit quality as well as one of the molecular signals regulating fruit maturity and aging. Sugar also plays important roles in regulating physiological functions, metabolism, gene expression, providing energy and substrates to plant growth, development and senescence (Halford and Paul, 2003; Steeves and Sussex, 1989; Dangl et al., 1995). It had been demonstrated that the synergies between sugar and ABA can accelerate leaf senescence and significantly increase the sugar content in aging leaves under drought stress (Wingler and Roitsch, 2008). Various genes regulated by soluble sugars may also accelerate leaf aging under the action of hexokinase, causing changes in various metabolic pathways (Xiao et al., 2000). Moreover, certain effects of sugar on fruit aging have been reported previously. At temperatures below 30 °C, the sugar accumulation in banana sarcocarp causes degradation of chlorophyll (Yang et al., 2009).

As primary metabolic products, organic acids play a regulatory role in plant growth and development also. Organic acids are metabolically active solutes in cellular osmoregulation and surplus cationic balance, acting as key components

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in response to nutritional deficiencies, metal ion accumulation and plant–microorganism interaction (López-Bucio et al., 2000). Organic acids can also enhance fruit resistance to diseases. Soaking fruits and vegetables in organic acids to lower the pH can inhibit both the growth of microorganisms and oxidation during storage at low temperature, which significantly extends the storage life of fruits (Rani, 2009). A set of postharvest properties of jujube (*Ziziphus jujuba* Mill. cv. Dongzao) fruit could be altered when treated by exogenous oxalic acid, including reducing ethylene production, delaying aging, slowing fruit reddening, decreasing the ethanol content, and enhancing the resistance to postharvest pathogenic microorganisms (Wang et al., 2009).

Sucrose, fructose and glucose are the primary soluble sugars, and citric and malic acid are the main organic acids in mature citrus fruit (Shimada et al., 2006; Vandercook, 1977). Citrus fruit has two anatomical parts, the endocarp which is also called the pulp, and the pericarp or peel (Goudeau et al., 2008). The pulp is also known as the juice sac and contains abundant soluble sugars and organic acids. The pericarp is composed of the flavedo and albedo, both of which are low in soluble sugars and organic acids contents. During postharvest storage, fruit pericarp usually consumes substrates during respiration, and for supplying energy to cope with resistance to microorganisms and various stresses, which ultimately results in the nutrient loss and quality degradation, which is unacceptable for consumers.

The postharvest biological properties of stored citrus fruits remain largely unknown; background information on the variations of soluble sugars and organic acids in postharvest stored citrus fruits is very limited. Pumelo is a storable fruit with thick pericarp (1–2 cm) and long storage life (5–8 months), which can be used as an ideal material to study the storage properties of citrus fruits as well as substance transport and redistribution between different tissues during fruit storage. In this study, the variation patterns of soluble sugars and organic acids in tissues were analyzed in various cultivars: Shatian pumelo (*Citrus grandis*, an ancient pumelo cultivar), Guanxi pumelo (*C. grandis*, seedless cultivar with mutated bud) and Hirado Butun pumelo (HBP; *C. grandis* × *Citrus paradisi*), which were expected to provide theoretical information regarding citrus storage practices.

2. Materials and methods

2.1. Fruit sample preparation

Matured (sugar/acid ratio over 8.0) HBP, Shatian pumelo were harvested from Changyang, Hubei Province, and Guanxi pumelo were obtained from Pinghe, Fujian Province. Fruits with normal size and color and without damage or insect infestation were chosen for the storage experiments. Three hundred fruits of each cultivar were stored at ambient temperature (10–16 °C) with a relative humidity of 85–90%. Each fruit was uni-packed with a 0.01 mm plastic film to prevent water loss; fruit samples were taken every 20 days; 15 fruits were collected for each cultivar (3 replicates, 5 fruits for each replicate). The fruit was separated into flavedo, albedo and pulp using a sharp scalpel, and then quickly frozen with liquid nitrogen and stored at −80 °C prior to use. Sampling and analyes were repeated for the three successive growing seasons of 2007, 2008 and 2009.

2.2. Organic acid analysis with high-performance capillary electrophoresis

Organic acids were measured using a Beckman P/ACE MDQ capillary electrophoresis apparatus (Beckman Coulter, Fullerton, CA, USA) with an uncoated fused silica capillary (Yongnian Optical Fiber Company, Hebei, China). Sample preparation and analysis procedures were performed following the method of Chen et al. (2006) with slight modifications. Each frozen sample was ground to a fine powder in 15 ml 80% ethanol prior to the high-performance capillary electrophoresis (HPCE) analyses, with three replicates. The mixture was incubated at 80 °C for 15 min, and then treated with ultrasonic sound for 30 min before it was centrifuged at 18,000 × $g\,min^{-1}$ for 15 min at 4 °C. The supernatant was diluted to 25 ml with 80% ethanol. After shaking, 2 ml of the sample was freeze-dried and then dissolved in 1 ml ddH_2O. For HPCE analysis, the four main organic acids, citric, malic, aconitic and fumaric acid, were separated in a phosphate buffer (100 mM Na_2HPO_4, 0.5 mM CTAB, pH 7.0) and their concentrations were determined by checking the UV absorption at the wavelength of 200 nm. Parameters for separation and identification of samples by HPCE were optimized. The injection pressure was set to 0.5 psi for 3 s. Separation was performed at 12 kV and 20 °C. All solutions and separation buffers were degassed by ultrasound for 10 min and filtered with a 0.22 μm filter prior to use.

2.3. Soluble sugars analysis with gas chromatograph

Soluble sugars were analyzed with a 7890A gas chromatograph (Agilent Technologies Co., Ltd., Wilmington, DE, USA), according to Bartolozzi et al. (1997). One gram of each freeze-dried sample was used for analysis. Each experiment was carried out in triplicate ($n = 3$).

2.4. Statistical analysis

Three replicates of each sample were carried out ($n = 3$). The results are expressed as means ± SE. Statistical comparisons were made by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparisons test. Differences were considered as significant at $P < 0.05$.

3. Results

3.1. Changes of organic acid concentrations in the pulp of three pumelo cultivars during storage

Concentrations of four main organic acids (citric, malic, aconitic and fumaric acid) in the pulp of three pumelo cultivars were measured by HPCE. The highest acids concentration was found in HBP, followed by Guanxi and Shatian pumelo. In the three pumelo cultivars, the citric acid concentration was highest, followed by malic, aconitic and fumaric acid. During the storage phase of 100 days post harvest (DPH), changes in organic acid concentrations in the pulp of three cultivars showed inconsistent trends. As Fig. 1A shows, organic acid concentration in the pulp of HBP decreased steadily until 60 DPH, and then increased, reaching a peak value of 8.49 $mg\,g^{-1}$ at 80 DPH. After this time, it decreased again, and the variations in concentrations of the four organic acids showed similar patterns. Compared with the earlier stage of storage, the total concentration of organic acid decreased significantly during the later stage, from 10.40 to 3.83 $mg\,g^{-1}$. Concentrations of the four organic acids in the pulp of Guanxi pumelo revealed significantly different patterns (Fig. 1B); the citric acid concentration increased from 3.23 to 6.81 $mg\,g^{-1}$; malic acid concentration increased at first, then decreased slowly to a level slightly higher than that at the earlier stage of storage. The total concentration of organic acids in the pulp of Guanxi pumelo showed an increasing trend from 3.43 to 6.96 $mg\,g^{-1}$. Organic acid concentrations in the pulp of Shatian pumelo changed in a similar pattern to that of HBP (Fig. 1C). Overall, the four organic acid concentrations showed the same changing