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Metabolic profiling and quality assessment during the postharvest of two tangor varieties subjected to heat treatments

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ABSTRACT

Citrus growth and export are one of the most important agroeconomical activities in regions with Mediterranean-like climates. Among them, tangors in particular are appreciated by their sweet taste and delicate palatability. The current study describes the analysis of the metabolome of two tangor (*Citrus reticulata* × *C. sinensis*) cultivars, Murcott and Ellendale, broadly cultivated in Argentina and other countries, after heat treatment and postharvest storage. This investigation intended to shed light on the biochemistry behind some observed differences in the fruit of both cultivars, such as a distinctive response to cold storage. The differential response to heat treatments and their effectiveness for preventing fungal infection was also evaluated. Metabolite profiling carried out using several chromatographic techniques and differential methods allowed to comprehensively compare the levels of sugars, organic acids, amino acids, polyalcohols, phenylpropanoids and phospholipids among varieties. It was found that Ellendale possess lower content of turanose and melibiose, higher levels of putrescine, unsaturated fatty acids, ribonic and propionic acids, as well as a remarkably higher level of hesperidin than Murcott. After heat treatment, Ellendale was less prone to pathogen development during storage than Murcott. The results, analyzed in terms of the differential metabolome response between the two varieties, hint at a better competence, boosted by heat treatment, of Ellendale to withstand biotic and abiotic stress conditions.

1. Introduction

Ellendale and Murcott are two tangor (*Citrus reticulata* Blanco) varieties of different origin. Both are hybrids between tangerine and orange, and are thus called tangor. Ellendale arose as a chance seedling near the Burrum River (Queensland, Australia) in 1878 (Ross, 1949), for which it is now considered to be a natural tangor (Hodgson, 1967). Ellendale is one of the most cultivated varieties in Argentina and is mainly assigned to exportation due to its excellent quality. It is a late variety which reaches optimum maturity from July to August in the Southern hemisphere. The origin of Murcott is unclear. It probably arose in a USDA plantation and by 1922 several trees were growing on the place formerly owned by Charles Murcott Smith, in Florida (U.S.A.). It is also known as Smith in Argentina or Honey in the U.S.A. (Hodgson, 1967). Murcott is also a late variety with almost the same harvesting period of Ellendale. In comparison, Murcott fruits have lower acidity content and are perceived as sweeter.

Export to distant markets stress the need for good postharvest management to ensure that fruit external and internal quality traits are conserved. Storage at low temperatures helps to reduce vegetables decay, but tropical and subtropical fruit as citrus, are prone to chilling injuries (Mulas and Schirra, 2007). With the aim to apply environmental-friendly technologies, heat treatments (HT) have been employed to improve fruit behavior during prolonged cold storage (Nafussi et al., 2001; Perotti et al., 2011, 2015; Yun et al., 2012, 2013; Lafuente et al., 2017) and their effects in mitigation of chilling injuries has been recently reviewed (Aghdam and Bodbodak, 2014). This is especially important in the case of mandarins and its hybrids, which tend to develop off-flavors much more rapidly than other citrus varieties. Most of these problems are associated with increases in juice ethanol and acetaldehyde or changes in acidity that alter the palatability and aroma (Cohen et al., 1990; Hagenmaier, 2002; Tietel et al., 2011). The mechanisms involved in the protection that HT confer are related to effects on cellular membrane integrity, accumulation of heat shock proteins, enhancement of sugar and antioxidant metabolism, among others (Sanchez-Ballesta et al., 2003, Lafuente et al., 2017). In

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mandarin, HT has been found to decrease carotenoid content (Matsumoto et al., 2009); in oranges and lemon HT caused an increase of antioxidant enzyme activities (Bassal and El-Hamahmy, 2011, Safizadeh et al., 2007). A transcriptomic based model of chilling tolerance in heat conditioned grapefruit, include up- and down-regulation of specific transcription factors, modification of lipid metabolism, accumulation of sucrose and repression of the expression of particular hormones and secondary metabolites pathways (Maul et al., 2008). More recently, results from Lafuente et al (2017) found that the accumulation of members from the WRKY transcription factors in heat conditioned Fortune mandarins was behind the gain of chilling tolerance. They also found that lipid degradation was repressed, while antioxidant and stress related metabolisms were enhanced.

Metabolite profiling has been widely used as a method to study plant responses to stress conditions. In fact, the abundance of some phenylpropanoids changed and three antimicrobial compounds were identified in *Penicillium* sp.-elicited oranges (Ballester et al., 2013). After natural frost, Valencia oranges suffered several changes in carbohydrate, iron and secondary metabolisms (Perotti et al., 2015). The nature and content of nutritionally valuable compounds present in kumquat has been found to be highly dependent on storage conditions (Schirra et al., 2007). The main goal of this work was to characterize the metabolic changes that occur during postharvest storage of the two tangor varieties exposed to biotic and abiotic stress. It is also pursued to advance towards a metabolite-based characterization that allows a comprehensive view of the basic molecular traits that act as the biochemical signature of the quality attributes of citrus fruits.

2. Material and methods

2.1. Plant material

Assays were conducted with 2 tangor varieties (*C. reticulata* cv `Ellendale´ and `Murcott´) grown in commercial orchards supervised by the Estación Experimental Agropecuaria Concordia del Instituto Nacional de Tecnología Agropecuaria (EEAC-INTA), in Concordia, province of Entre Ríos, Argentina, harvested at full maturity in September 2014. The temperature record in the 30 days previous to the harvest showed typical winter values with no meteorological frost events (Supplementary table 1, data can be accessed at www.inta.gob.ar/documentos/agosto-2014-resumen-agroclimatico-de-concordia). Immediately after harvest, fruit were manually selected for uniformity of color and size. Flavedo tissue (epicarp) were separated, frozen in liquid nitrogen and stored at −80 °C for further experiments.

2.2. Heat treatments, sampling procedure and Penicillium digitatum infection

Heat treatments (HT) consisted of exposure to 37 °C and 97% relative humidity for 24 hours, followed by 24 h at 20 °C. Controls lacked the HT and were left at 20 °C, 90% relative humidity during 24 h (Fig. 1). Samples were stored at -80 °C until analysis.

To measure rot incidence, 3 replicates of 30 fruit each were done, and treatment was performed as described in Ballester et al. (2013). Briefly, fruit were wounded in the equatorial zone with a piercing element (2 mm long, 1 mm wide) imbibed in a solution containing 10^6 *P. digitatum* conidia. These samples were left 24 h at 20 °C, 90% relative humidity until HT was applied as before. The samples were left for 30 d at 5 °C, after that period, the percentage of rot fruit was calculated. Conidia were prepared basically as described by Smilanick et al. (2005): *P. digitatum* was cultivated in potato glucose agar for 7 days at 25 °C. Conidia were extracted by flooding with sterile water containing 0.005% (v/v) Tween, filtered 4 times through cheesecloth and diluted to an absorbance of 0.1 at 420 nm, which corresponds approximately to 1×10^6 spores/ml.

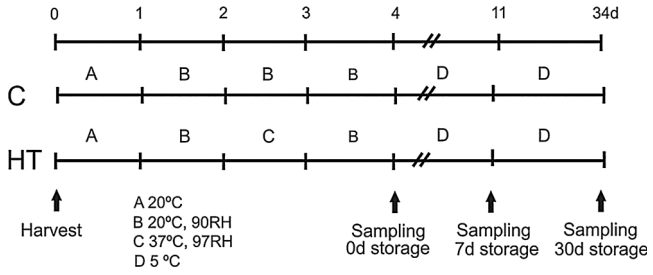

Fig. 1. Schedule of the experimental design. Solid lines indicate the duration of the incubation period in days. Letters A, B, C or D refer to different temperature and humidity conditions. Solid arrows below indicate time of sampling at different storage period after harvest. C: control fruit; HT: heat treated fruit.

2.3. Determination of internal quality parameters

Fruit were squeezed and the resulting juices were used for the following determinations. Five fruits were used per replication (3 replication samples). The total soluble solid content in the juice was determined with a digital refractometer and expressed as % w/v. The titratable acidity was measured by titrating with 0.1 N NaOH to pH 8.2. The results are expressed as the percentage of anhydrous citric acid because this is the predominant organic acid in citrus. The maturity index was evaluated as the ratio of total soluble solids: titratable acidity. Juice was extracted from individual fruit (at least 5 fruit per replication, 3 replication samples), and the juice content was expressed as a percentage of fresh fruit weight.

Ethanol and acetaldehyde contents in juice were determined by gas chromatography analysis of juice headspace using a gas chromatograph (Shimadzu Mod. GC17A; Shimadzu Corp., Kyoto, Japan) with a Supelco Omegawax 250 column (30 m × 0.25 mm internal diameter) (Supelco, Bellefonte, PA). This experiment was carried out on 5 fruit per replication (3 replication samples).

2.4. Primary metabolite extraction, derivatization and analysis

Samples were prepared as described by Perotti et al. (2011) with slight modifications. Frozen flavedo tissue (300 mg) from 3 different fruit (3 replication samples) was powdered with mortar and liquid nitrogen and transferred to glass tubes with 4.2 mL of cold methanol (−20 °C). After shaking in a vortex, 75 µg of ribitol were added as internal standard to be used for the relative quantification of metabolites. The resulting extracts were incubated at 70 °C for 15 min, with periodic agitation. Subsequently, 1.5 mL of chloroform were added, followed by incubation at 37 °C for 5 min. Finally, after addition of 3 mL of water, extracts were centrifuged in a refrigerated microcentrifuge for 15 min at 2200 × g. A volume of 450 µl of the polar phase was transferred to microcentrifuge tubes and dried in a vacuum centrifuge (CentriVap, Labconco) until complete evaporation, leaving a colored pellet. Samples were stored at −80 °C until derivatization.

Samples were thawed, dried in a vacuum centrifuge for 30 min to ensure no liquid was present. Then, 30 µL of 20 mg/mL methoxyamine in pyridine were added. Tubes were vigorously shaken and incubated at 37 °C for 90 min. Finally, 45 µl of *N*-methyl-*N*-trimethylsylil-trifluoroacetamide (MSTFA) were added to each tube and incubated at 37 °C for 30 min. Chromatographic runs were performed by injecting 2 µL of derivatized sample in a 30 m long, 0.25 mm thick VSF MS capillary column using an automatic system (Varian Inc.) coupled to a ThermoQuest mass spectrometer. Data were collected and analyzed using the Lab Solution software (Shimadzu). Spectra obtained from chromatographic runs were used to analyze the individual peaks that showed a significant value. Areas underneath each peak were calculated and expressed as the area relative to the internal standard, ribitol. Data were revised using the online software Mass Spectra & Retention Time Index (MSRI) (The Comprehensive System Biology Project - CSB,