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Influence of postharvest treatments on qualitative and chemical parameters of Tarocco blood orange fruits to be used for fresh chilled juice

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ABSTRACT

Tarocco "Sant'Alfio" is a late ripening blood orange cultivar. Blood oranges are more and more appreciated from consumers for their high nutraceutical value due to the presence of bioactive compounds including vitamin C, polyphenols, flavonoids and hydroxycinnamic acids.

The aim of this work is to set up a reliable protocol for postharvest storage of the very-late Tarocco "Sant'Alfio" orange to prolong the availability of this product in the market to be used for fresh chilled orange juice production.

Fruits were subjected to three storage treatments (20 days at 1 °C plus 50 days at 4 °C; 70 days at 4 °C; 70 days at 20 °C). The results indicate that cold treatments, in particular at 4 °C constantly, can extend Tarocco "Sant'Alfio" shelf life enhancing total anthocyanin content. The defined protocols allow prolonging market availability of a high value product and could induce relevant benefits for the citrus industry and consumers.

1. Introduction

Sweet oranges (*Citrus sinensis* L. Osbeck) are usually categorized into two groups according to the peel and pulp color: blonde and pigmented (blood) oranges. Blood oranges are mainly cultivated in Sicily (Italy) where they are widely spread and play a pivotal role in local citrus industry (Barreca, Gattuso, Laganà, Leuzzi, & Bellocco, 2016). It has been demonstrated that Sicilian typical environmental conditions (namely night/day remarkable thermal excursion) exert an important role on pigment biosynthesis and accumulation on fruits of selected genotypes (Rapisarda & Giuffrida, 1992; Butelli et al., 2012), thus improving nutritional value and consumer acceptance. The most important blood orange cultivars are Moro, Tarocco and Sanguinello and among these, Tarocco is appreciated for fresh consumption, especially for its easy peelability and for the low brix-acidity ratio which attenuate its sweet taste (Rapisarda & Russo, 2000). Moreover, during the last thirty years, Italian researchers have isolated a number of lines derived from old Tarocco varieties, that, on the whole, allowed widening its marketing calendar from December till May (Tribulato & La Rosa, 1994). The secondary metabolic pool of blood orange cultivars is well known; it includes flavanone glycosides,

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which can be also considered as markers of the genus Citrus (Siracusa & Ruberto, 2014, and references therein), several hydroxycinnamic acids and their conjugates (Rapisarda, Carollo, Fallico, Tomaselli, & Maccarrone, 1998; Rapisarda, Fabroni, Peterek, Russo, & Mock, 2009; Fallico, Ballistreri, Arena, Brighina, & Rapisarda, 2017), flavone glycosides (Barreca et al., 2016) and anthocyanins, to which their typical red color is ascribable (Lee, 2002; Dugo, Mondello, Morabito, & Dugo, 2003; Hillebrand, Schwarz, & Winterhalter, 2004; Kelebek, Canbas, & Selli, 2008). Anthocyanin biosynthesis and accumulation mechanisms have been studied so far by several authors (Maccarrone, Maccarrone, & Rapisarda, 1985; Maccarrone, Rapisarda, Fanella, Arena, & Mondello, 1998; Fabroni, Ballistreri, Amenta, & Rapisarda, 2016). In all the pigmented varieties the most represented anthocyanins are cyanidin 3-glucoside and cyanidin 3-(6″- malonyl)glucoside (Maccarrone et al., 1998). The biosynthesis of free anthocyanins follows the flavonoid pathway and involves the expression of structural genes (responsible for enzymes directly implicated in all the metabolic reactions) and of their regulatory genes (Lo Piero, 2015, and references therein). Besides anthocyanins, other polyphenols have been investigated for their importance as quality assessment markers (Peleg, Naim, Rousseff, & Zehavi, 1991; Rapisarda et al., 1998; Siracusa & Ruberto, 2014). In comparison to blonde cultivars, blood oranges are richer in hydroxycinnamates (Rapisarda et al., 1998; Arena, Fallico, & Maccarrone, 2001); on the other way the presence of anthocyanins in their metabolic pool implies an higher susceptibility to chilling injury (CI), with symptoms, as peel pitting of various sizes and shapes, appearing after 2–3 weeks of storage at temperatures below 8 °C (Pratella, Tonini, & Cessari, 1969). Recently, cold treatment has been considered on different pigmented fruit commodities including cherry (Özkaya et al., 2015) and pomegranate (Palma, Continella, La Malfa, Gentile, & D'Aquino, 2015), and blood oranges, also for flesh pigmentation enhancement (Crifò, Puglisi, Petrone, Reforgiato Recupero, & Lo Piero, 2011). Cold treatment is also considered a reliable procedure to accomplish quarantine regulations for citrus fruit to be exported to the United States and Japan. In particular, treatment protocol T107-a (APHIS, 2006) including storage at 1.1 °C, 1.67 °C or 2.2 °C for 14, 16 or 18 days, respectively, has been proven as effective against Mediterranean fruit-fly (*Ceratitis capitata* Wiedemann).

The renewed nutritive values of orange juice (Grosso et al., 2013; Zhuo, Wanpeng, Yan, Chao, & Zhiqin, 2016) and the request of nutraceuticals by consumers are pushing towards an increase of consumption of fresh-commercial juice instead of those of other categories (from concentrate or not from concentrate). Furthermore, anthocyanins stability and nutraceutical properties are depleted by thermal processing such as pasteurization (Lo Scalzo, Iannoccari, Summa, Morelli, & Rapisarda, 2004; Cassano, Marchio, & Drioli, 2007; Baldwin et al., 2012; Bai et al., 2013). For this reason, the extension of raw fruits shelf life could be a strategy in order to ensure the availability of fruits to be used for fresh chilled juice production during summer season.

In such a context, the aim of this work was to evaluate the effects of different postharvest storage conditions on qualitative and compositional traits of one of the latest ripening Tarocco lines, namely Tarocco "Sant'Alfio", in order to extend raw fruits availability.

2. Material and methods

2.1. Plant material

Tarocco "Sant'Alfio" sweet orange [*Citrus sinensis* (L.) Osbeck] fruits were picked from plants grafted onto sour orange and grown in a commercial orchard located in south east Sicily (Italy) on the mountainsides of the Etna volcano (37°16′59″N; 14°53′09″E). Fruits were harvested at commercial maturity, at the end of April, and immediately transported in laboratory.

2.2. Treatment and storage conditions

Fruits were rinsed in 3% sodium hypochlorite water solution for 10 min to reduce surface contamination and then dried with blotting paper. For each treatment 50 kg of fruits were used, generating three replicate samples of 15 kg of oranges, randomly packed in 3 rigid boxes, each representing one replicate.

Three different treatments were evaluated. Specifically, a first group of fruits was stored at 1 ± 1 °C for 20 d and then at 4 ± 1 °C and 90–95% relative humidity (RH) for 50 d (T1); a second group was stored at 4 ± 1 °C and 90–95% relative humidity (RH) for 70 d (T2); the third group of fruits was stored at ambient condition (20 ± 1 °C and 70 ± 5% RH) and used as control sample (CK). Six fruits (with three replicates) were analysed in each treatment at 0, 20, 35, 48, and 70 days after harvest and used for morphological and chemical parameters determination. Physiological disorders was evaluated on each box during holding of conditioned and control samples every week after harvest.

2.3. Morphological and physicochemical parameters determination

Incidence of decay was expressed as percentage of moldy fruits on the total number of fruits examined.

Fruit height, equatorial diameter and weight, rind thickness, texture and color parameters were recorded on each fruit before juice extraction. Fruit height and equatorial diameter (mm) were measured with an electronic digital slide gauge (Mitutoyo) with 0.01 mm accuracy; the fruit weight was taken using an electronic balance (Sartorius Model BL-600) with an accuracy of 0.1 g.

Peel color was recorded on two opposite points of the equatorial region of each fruit using a Minolta CR-400 chroma-meter according to the international CIE L*, a*, b* values, where L* indicates lightness, a* indicates chromaticity on a green (−) to red (+) axis, and b* chromaticity on a blue (−) to yellow (+) axis. Results were expressed as citrus color index (CCI = a*1000/L*b), widely used in the citrus industry as maturation index (DOGV, 2006).

Fruit firmness was measured by a Stable Micro System TA-XT2 Texture Analyzer texture analyser (Godalming, UK) equipped with a flat compression plate; each fruit was compressed in two opposite site and the resistance to compression of 10 mm s^{-1} was expressed in Newton (N).

For physicochemical and chromatographic analyses, fruits were individually squeezed with a commercial juice extractor (Kenwood Citrus Juicer JE290) and the pooled juice of six fruits per replicate was analysed. Total Solid Soluble (TSS) content was determined using a digital refractometer (Atago CO., LTD, model PR-32α) and results expressed as °Brix. Titratable acidity (TA) was determined by potentiometric titration (Hach, TitraLab AT1000 Series) of the juice with 0.1 N NaOH up to pH 8.1 according to the AOAC method (AOAC, 1995) and results were expressed as g of citric acid equivalent L^{-1} juice.

Vitamin C (l-ascorbic acid) was determined using an automatic titration apparatus (702 SM Titrino, Metrohm, Herisau, Switzerland) with 0.001 M I_2 and results were expressed as g L^{-1}.

2.4. HPLC/DAD and HPLC/ESI/MS analyses

All solvents and reagents used in this study were high purity laboratory solvents from VWR (Milan, Italy); HPLC grade water and acetonitrile were also obtained from VWR. Cyanidin 3-*O*- glucoside, caffeic acid, chlorogenic acid, p-coumaric acid, ferulic acid