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Effects of preharvest applications of natural antimicrobial products on tomato fruit decay and quality during long-term storage

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ABSTRACT

The effects of preharvest sprays of Thyme essential oil, Propolis and Chitosan on postharvest quality and decay of a “long-storage” tomato, called “Vesuviano”, stored at room temperature for 120 days, were investigated. Postharvest fruit quality [number of swollen-healthy fruits (SF), withered-healthy fruits (WF) and rotten fruits (RF)], organoleptic-related indexes (dry matter, soluble sugars, organic acids, volatiles) and health-related compounds (total carotenoids and phenols) were investigated at 40, 80 and 120 days post-harvesting (T40, T80, T120). Propolis and Chitosan were able to reduce rotten fruits starting from T80, while the effect of Thyme was evident as early as T40. Furthermore Chitosan delayed fruit senescence (as expressed by SF/WF ratio) during the long-storage period. All treatments did not affect the overall postharvest quality, nevertheless some compounds (such as total soluble sugar for Chitosan and Thyme; total carotenoids and flavonols for Chitosan; total organic acids, 2-(E)-hexenal, 2-isobutylthiazole and terpenes for Propolis), were better retained than the control during postharvesting period. Among the three natural fungicides, Chitosan was most effective in reducing fruit senescence, maintaining a good quality of the fruits over a long-term.

1. Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most produced and extensively consumed vegetable crops in the world (Sacco et al., 2015). Fresh fruits and tomato-based foods provide basic organoleptic features, as well as a wide variety of nutrients and health-related phytochemicals. Sugars and organic acids include the majority of the total dry matter content of tomato fruit. The most abundant sugars are fructose and glucose, while major organic acids are citric and malic, with predominance of the first acid (Davies and Hobson, 1981). Earlier studies have shown that the level of sugars and acids affect not only tomato taste attributes, but also the overall flavour (Hobson and Bedford, 1989). A relevant contribution to tomato flavour is also given by volatiles: hexanal, 2(*E*)-hexenal and 2-isobutylthiazole are considered as characteristic tomato compounds (Dirinck et al., 1976). Moreover, terpenes have been demonstrated to contribute to product's quality (Baldwin et al., 2000). The most important health-related compounds in tomato fruit are carotenoids with lycopene being the most representative (Shi and Maguer, 1999). Epidemiological studies have highlighted a strong association between consumption of carotenoids-rich foods and prevention of different cancers such as cervical, breast, colon, prostate, rectal and stomach (Dorais et al., 2008). Phenolic compounds, such as naringenin chalcone, quercetin-type flavonols and hydroxycinnamic acids, are another important class of phytochemical compounds of tomato fruit (Giovannelli et al., 1999). Their potential benefit for human health, likely due to the antioxidant activity, is widely acknowledged (Siracusa et al., 2012).

Despite benefits that can be derived from the crop, postharvest losses make its production in most parts of the world unprofitable. Postharvest losses in tomatoes can be as high as 25–42% globally and are due to physiological disorders, physical injury and fungal infections (Arah et al., 2015). Fruits are susceptible to attacks of several

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pathogenic fungi [such as *Alternaria alternata* (Fr.:Fr.) Keissl., *Botrytis cinerea* (Pers), etc.] making them unsuitable for consumption by producing mycotoxin, in addition to causing rots (Ibrahim and Al-Ebady, 2014). Although public opinion strongly demands for a reduction of synthetic agents, fungicides are still the primary means of controlling postharvest diseases. Negative effects of chemical fungicides call for alternative products that reduce losses due to postharvest decay. Among naturally derived substances, *i.e.* GRAS (Generally Recognized as Safe), essential oils, propolis and chitosan were recently considered with the aim to controlling biological spoilage and extending the storage life of different fresh commodities (Tripathi and Dubey, 2004).

Plant-derived essential oils are considered non-phytotoxic compounds and are potentially effective as natural pesticides for crop protection (Pane et al., 2013). They contain complex mixtures of secondary metabolites, which are biologically active, endowed with antimicrobial, allelopatic, antioxidant and bioregulatory properties (Antunes and Cavaco, 2010).

Propolis is a natural resinous substance obtained from leaf buds and bark of poplar and conifer trees. It has antibiotic, antibacterial and antifungal activity (Tripathi and Dubey, 2004). Application of propolis has shown positive effects on reducing decay and extending the shelf life of different vegetables and fruits (Yang et al., 2016).

Chitosan is a cationic polysaccharide produced by alkaline N-deacetylation of chitin and it has been reported to have plant protective and antifungal properties on different crops (Pichyangkura and Chadchawan, 2015).

The efficacy of postharvest applications of different natural antimicrobial substances on reducing postharvest losses have been documented in earlier studies (Aminifard and Mohammadi, 2012; Reddy et al., 2000; Ordóñez et al., 2011). Conversely, studies regarding the preharvest use are reported for few fresh commodities excluding tomato crop (Romanazzi et al., 2002, 2012, 2013; Saavedra et al., 2016; Tezotto-Uliana et al., 2014; Meng et al., 2008). An improved investigation on preharvest uses depend on varieties or landraces belonging to each fruit or vegetable species, since they differ in term of shelf lives extension and storage condition (temperature, relative humidity) can be very diversified. Therefore the effectiveness of GRAS substances adopted to control postharvest decay and quality can be severely affected by varying background conditions.

Several cherry-like tomato landraces showing high drought tolerance are traditionally cultivated in Mediterranean countries, and especially in southern Italy, under non-irrigated conditions. Peculiar textural properties, such as high firmness and thickness of skin, allow an extended shelf life of the fruits, which after the harvest are stored in the typical hung-shaped appearance for 3–4 months under no-conditioned atmosphere ("long-storage tomatoes") (Siracusa et al., 2012; Mercati et al., 2015). The most important and profitable "long-storage" landrace is cultivated on the slopes of the Vesuvio volcano (Campania region) and is called "Vesuviano" or "Pomodorino del piennolo del Vesuvio" (labeled as PDO − Protected Designation of Origin; Reg. 1238, 2009; Ercolano et al., 2008, 2014). This high-value product is commercialized throughout the Italian peninsula and in other European countries starting from winter to early spring, when high-quality fresh tomatoes are not available on the markets. However, despite the prolonged shelf life, unprofitable fruit rots and quality decay are currently observed during the typical storage of "Vesuviano" landrace. Therefore the adoption of means to limit these losses are highly desirable.

Based on these premises, the objectives of this study were (i) to examine the effects of preharvest spraying of propolis, chitosan and thyme essential oil for controlling natural decay and (ii) to evaluate postharvest quality during the typical prolonged storage at room temperature by measurement of dry matter, soluble sugars, organic acids, by the main health-related compounds (phenols and total carotenoids), and evaluating volatile substances both for tasting (hexanal, 2(*E*)-hexenal, 2-isobutylthiazole and total terpenes) and for fermentation-senescence markers (ethanol).

2. Material and methods

2.1. Field trial, treatments and storage conditions

The study was performed adopting a previously described accession of "Vesuviano" landrace (PV-ISCI 10) (Ruggieri et al., 2014). Tomato plants were grown in open field, according to the traditional practices, on the slopes of Vesuvio volcano (Massa di Somma, 40° 50′ 0″ N, 14° 22′ 0″ E, 175 m a.s.l.). Transplant was performed in single rows with a density of 5.0 plants m^2. A single drip irrigation was applied in support of rainfall along the growing season (115 mm). This experiment included three preharvest treatments: Chitosan (Chitoplant®, Agritalia − Villa Saviola, Italy), Propolis (Propoli®, Serbios − Badia Polesine, Italy) and Thyme (*Thymus vulgaris* essential oil, A.C.E.F. − Fiorenzuola d'Arda, Italy). These substances were diluted by water to give final concentrations of 0.3%, 0.2% and 1% (water emulsion) respectively, and were compared to untreated control (water). The commercial product Chitoplant® was an aqueous solution containing 95% of chitosan (47–65 kDa), 2.5% of boron and 2.5% of zinc; Propoli® was a propolis glycolic extract with a flavonoid content (expressed as galangin) of 20 mg ml^{-1}, while Thyme was *Thymus vulgaris* essential oil containing 57.4% of thymol and 2.8% of carvacrol. The above-mentioned natural antimicrobial substances were spread on the plants (at 5, 15, 25 and 35 days before the harvest) until all fruits were wet to runoff.

Field trials were carried out in a completely randomized experimental design with three replicates and 30 plants for each replicate. At harvest (July 31, 2013), successively mentioned as T0, ripe bunched fruits were harvested taking care not to detach them from peduncles. For each experimental plot, 8 kg of fruits (320 in number, approximately) were selected based on uniformity of size and colour and absence of physical injury or lesions caused by pathogen. These quantities were subdivided in two wooden boxes, each containing 4 kg approximately of fresh product, used for quali-quantitative market assessment and chemical-physical analysis. The overall samples (without any packaging) were then transferred in an unconditioned and well-ventilated shed up to 120 days after harvest, where temperature and relative humidity were recorded, every 60 min, by a wireless data logger (EL-USB-2 model, Lascar Electronics Ltd. − United Kingdom) (Supplementary Fig. 1).

2.2. Postharvest decay assessment

In order to evaluate the decay rate, both treated and untreated fruits of each experimental plot were visually observed at 40 (September 10, 2013), 80 (October 20, 2013) and 120 (November 30, 2013) days post harvest (thereafter mentioned as T40, T80 and T120). At these time points fruits were classified in three categories: swollen-healthy fruits (SF) (ripe fruits without abiotic/biotic damages and showing high firmness), withered-healthy fruits (WF) (wrinkled/senescent fruits without abiotic/biotic damages and showing low firmness) and rotten fruits (RF) caused by physiological or biological alterations (Supplementary Fig. 2). Number of fruits falling in these classes were recorded at each time point and the relative incidence (%) was expressed as cumulative number of each category on number of fruits at harvest (T0). RF were discarded each time as waste product, while WF and SF represented the commercial yield.

A preliminary report (Carrieri et al., 2016) provided technical details and specific analysis on post harvest decay microorganisms.

2.3. Analytical methods

2.3.1. Tomato samples

For each treatment, healthy tomato fruits were sampled in duplicate