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Research article

Postharvest responses of sweet cherry fruit and stem tissues revealed by metabolomic profiling

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ABSTRACT

Sweet cherry, a non-climacteric and highly perishable fruit, is usually cold-stored during post-harvest period to prevent senescence; therefore, metabolic profiling in response to cold storage in sweet cherry is of economic and scientific interest. In the present work, metabolic analysis was performed in fruit and stem tissues to determine the metabolic dynamics associated with cold storage in response to 1-methylcyclopropene (1-MCP), an ethylene-action inhibitor, and modified atmosphere packaging (MAP). Fruit (cv. Regina) following harvest were treated with 1-MCP and then cold-stored (0 °C, relative humidity 95%) for 1 month in the presence or in the absence of MAP and subsequently maintained at 20 °C for up to 2 days. Physiological analysis suggested that cold storage stimulated anthocyanin production, respiration rate and stem browning. Cherry stem exposed to 1-MCP displayed senescence symptoms as demonstrated by the higher stem browning and the lower stem traction force while MAP treatment considerably altered these features. The metabolic profile of fruits and stems just following cold storage was distinctly different from those analyzed at harvest. Marked tissue-specific differences were also detected among sweet cherries exposed to individual and to combined 1-MCP and MAP treatments, notably for amino acid biosynthesis. The significance of some of these metabolites as cold storage hallmarks is discussed in the context of the limited knowledge on the 1-MCP and MAP response mechanisms at the level of cherry fruit and stem tissues. Overall, this study provides the first steps toward understanding tissue-specific postharvest behavior in sweet cherry under various conditions.

1. Introduction

In recent decades, sweet cherry (*Prunus avium* L.) has become one of the most important non-climacteric fruits worldwide (Commisso et al., 2017; Mirto et al., 2018) that is greatly valued by consumers for its taste, color, nutritional value, and beneficial health properties (Ballistreri et al., 2013). However, sweet cherry postharvest life is relatively short due to their respiratory activity and susceptibility to rapid senescence, manifested by browning and drying of the stems, darkening of fruit color and shriveling (Chockchaisawasdee et al., 2016; Correia et al., 2017). Unlike other fleshy fruits, there are unique aspects of cherry fruit senescence (Wani et al., 2014). Cherries are harvested, cold-stored and marketed with their stem, which exhibits tissue specific physiological and metabolic differences to that of the edible part. For instance, cherry stem has, among others, a much thinner epidermis and cuticle layer than the fruit itself, resulting in a higher sensitivity to water and carbon dioxide losses (Sekse, 1996). Their resistance to water vapor transfer is much lower and stems could lose water up to eight-times faster than fruits (Linke et al., 2010). Consequently, stems metabolism seriously affects the whole cherry fruit senescence process. In contrast to the cherry fruit itself, almost no detailed studies on stem metabolic changes have been performed previously. Therefore, sweet cherry fruit can be a particularly advantageous model for studying the post-harvest responses of woody perennial fruits.

Post-harvest cold treatments are generally used to store sweet cherry properly (Tsaniklidis et al., 2017) but the mechanisms underlying the cold-dependent fruit post-harvest ripening are still relatively unknown for sweet cherries. It has previously been shown that several sweet cherry quality-related secondary metabolites seem to be regulated by cold exposure. For example, the level of phenolic compounds

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such as neochlorogenic acid, *p*-coumaroylquinic acid, chlorogenic acid, rutin, catechin and epicatechin, and the content of anthocyanins such as cyanidin-3-*O*-rutinoside and peonidin-3-*O*-rutinoside increased in several sweet cherry cultivars during cold storage (Gonçalves et al., 2004; Serrano et al., 2009; Goulas et al., 2015).

In addition to cold storage, several techniques to extend the postharvest life of cherries have been established. Particularly, modified atmosphere packaging (MAP) is commercially used to extend the market life of sweet cherries (Remon et al., 2004; Serrano et al., 2005; Chockchaisawasdee et al., 2016). The level of O_2 decreases while CO_2 increases inside the package due to respiration of the fruit and the increased CO_2 rate could, in turn, may have a profound impact of MAP on fruit metabolism (Habib et al., 2015). However, despite many commercial uses of MAP treatments, there is no published data on the effect of MAP on fruit metabolism. In various climacteric fruits, the pre-climacteric application of 1-methylcyclopropene (1-MCP), a potent inhibitor of ethylene perception due to its largely irreversible binding to ethylene receptors, has been reported to delay ripening and senescence (Minas et al., 2013). Evidence also suggests that 1-MCP might affect certain ripening-related processes and physiological disorders in non-climacteric fruit (Li et al., 2016). In parallel, it has been proposed that sweet cherry fruits may have the potential to biosynthesize ethylene (Ren et al., 2011). Although, these examples suggest that 1-MCP could shapes ethylene-associated responses in cherry fruit, it remains elusive how 1-MCP can influence the fruit metabolism.

The objectives of this study were dual: (1) to characterize the physico-chemical and metabolic shift that occur in the 'Regina' fruit and stem tissues following cold storage; and (2) to understand the tissue-specific metabolic modifications associated with 1-MCP and MAP application. The outcomes of this study could contribute to better understanding cherry postharvest behavior, thereby will ensure the development of effective practices for high-quality sweet cherry fruits.

2. Materials and methods

2.1. Fruit material, treatments and sampling process

Sweet cherries (cv.) 'Regina' were harvested from a commercial orchard (Pieria, North Greece) at physiologically mature stage. Fruits were randomly divided into two groups of 240 fruits each. Cherries of the first group were treated with 1-MCP (625 ppm) for 24 h at 0 °C. Immediately after 1-MCP treatment, the fruits of all groups were cold stored (0 °C and 95% RH) and enclosed or not in MAP for 1 month. Following cold period, fruits were transferred to room temperature (20 °C) and analyzed after 0 or 2 days.

2.2. Estimation of physiological disorders development

The subjective estimation of the stem quality was conducted according to Goulas et al. (2015) as referred; stem color: 0: was green with fresh appearance or less than 30%; brown, 1: was substantially green with 30–50%; brown, and 2: was substantially brown with less than 50% green. Physiological disorders, including shrivel (%) and pitting (%) were estimated as reported by Michailidis et al. (2017).

2.3. Sweet cherry postharvest characteristics

2.3.1. Ripening traits

Soluble solid concentration (SSC), titratable acidity (TA; malic acid, %) and ripening index (SSC/TA) were measured from 10 fruits per replication according to procedure described in detail elsewhere (Karagiannis et al., 2016).

2.3.2. Respiration rate

Respiration rate was measured at harvest (before cold storage) as well as at 0 and 2 days at room temperature after 1month of cold storage. For each treatment 3 batches of 10 fruits were weighed and placed into 2 L volume airtight jars for 60 min. CO_2 determination was conducted by withdrawing a 1 mL headspace gas sample from each airtight jar and injecting it into a gas analyzer (infrared gas analyzer; 280 Combo, David Bishop Instruments, Heathfield, UK), as detailed (Tanou et al., 2017).

2.3.3. Color parameters

External color was measured with a Minolta CR200 colorimeter (Minolta, Osaka, Japan) and the CIE (Commission International de l'Eclairage) parameters L^* (lightness), a^* (redness) and b^* (yellowness), C^* (chroma) and h (hue angle) were determined in 30 fruits per treatment, performing measurements at the equator at two opposite surfaces of each fruit (Goulas et al., 2015).

2.3.4. Total anthocyanin determination

Sweet cherry total anthocyanins were extracted with 80% ethanol +1% HCl, as described (Karagiannis et al., 2016). The anthocyanin content was estimated by the pH-differential assay at 520 and 700 nm. The final anthocyanin concentration was calculated as cyanidin-3-O-glucoside equivalent.

2.4. Textural properties

For instrumental analysis of the sweet cherry textural properties, stem removal and 15% fruit deformation, 30 fruits from each treatment were determined using a Texture Analyzer TA XT2i (Stable Micro systems, Godalming, Surrey, UK). For the stem traction force measurement, a tensile grip was used in order the stem to be removed with traction force; the fruit was placed beneath a plate having a hole in the middle (10 mm diameter) through which the stem was directed to the grip. Additionally, fruit firmness was estimated using a flat aluminum plate (75 mm diameter) connected to the analyzer; fruits were placed with their large dimension on the 'crisp fracture support ring' (Stable Micro systems). The force required for the 15% deformation of the fruit height was recorded; the speed of the compression plunger was 0.8 mm s^{-1}. Normalized firmness force was calculated as the ratio of the force required in order to be achieved 15% fruit deformation over the fruit large diameter (N mm^{-1}) (Goulas et al., 2015).

2.5. Primary metabolite profiling of sweet cherry tissues

Primary polar metabolites extraction was performed as described (Michailidis et al., 2017) at harvest as well as following cold storage (0 d at 20 °C). Frozen tissues (fruit and stem, 0.5 g), transferred in 2 mL screw cap tubes and 1400 mL of precooled (−20 °C) pure methanol was added. Subsequently, internal quantitative standard of adonitol (100 mL of 0.2 mg mL^{-1}) was added and solutions were incubated for 10 min at 70 °C. Supernatant was collected after centrifugation at 11.000*g*, 4 °C, 10 min and 750 mL precooled chloroform (−20 °C) plus 1500 mL dH_2O (4 °C) were added. Following centrifugation (2200 g, 4 °C, 10 min), the upper polar phase, transferred into a new 1.5 mL vial glass, which placed under constant nitrogen flow until drying. Dried residues were dissolved in 40 μL of 20 mg mL^{-1} methoxyamine hydrochloride for 2 h at 37 °C by gentle shaking and subsequently 70 μL of N-methyl-N-(trimethylsilyl) trifluoroacetamide reagent (MSTFA) were added and incubated for 30 min at 37 °C. Gas chromatography–mass spectrometry (GC-MS) analysis was carried out in Thermo Trace Ultra GC equipped with ISQ MS and TriPlus RSH autosampler (Switzerland). One (1) microliter (μL) was injected into the GC with a split ratio of 70:1. Separation was conducted on a TR-5MS capillary column 30 m × 0.25 mm × 0.25 μm. The injector temperature was 220 °C, ion source 230 °C and interface 250 °C. Constant flow rate of 1 mL min^{-1} was used for carrier gas. GC temperature program was carried out for 5 min at 70 °C, then increased to 260 °C (rate 8 °C min^{-1}), where it remained for 15 min. Mass range of m/z 50–600 was recorded, after