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Use of the index of absorbance difference (I_{AD}) as a tool for tailoring post-harvest 1-MCP application to control apple superficial scald

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ABSTRACT

The aim of this study was to better define postharvest strategies to preserve apple fruit quality during storage with particular attention on superficial scald control as the result of the interaction between 1-methylcyclopropene (1-MCP) treatment and fruit ripening stage. Ripening was no-destructively defined by the "index of absorbance difference" (I_{AD}) measured with a DA-Meter, a portable device based on visible/Near Infra-Red (vis/NIR) spectroscopy. Superficial scald incidence and total content of α-farnesene and conjugated triols (CTols), were assessed at two month intervals, among 6 months of cold storage (1 °C) for three consecutive seasons in two apple cultivars, 'Granny Smith' and 'Cripps Pink'. Results demonstrated the reliability of the I_{AD}, not just to assess fruit maturity, but also to predict scald incidence in both apple cultivars as a function of maturity and postharvest control strategy. Consequently, differential post-harvest treatments can be applied to single appropriate apple batches increasing storability and shelf-life, while reducing spoilage.

1. Introduction

Superficial scald is one of the main post-harvest physiological disorder affecting pome fruits (Watkins et al., 1995; Mattheis, 2008; Lurie and Watkins, 2012). In apple, the predominant scald symptom is represented by diffuse necrotic areas on the surface layers of the hypodermal cortical tissue cells, resulting in a browning coloration of the fruit skin, without, however, impacting the inner flesh tissues (Lurie and Watkins, 2012; Whitaker, 2004; Bain and Mercer, 1963). This physiopathy normally occurs after the re-establishment of room temperature conditions (20 °C) following a period of cold storage (at −1 to 4 °C; Watkins et al., 1995).

The physiological mechanisms leading to scald is still highly debated in apple, since the real aetiology of this phenomenon is not yet completely elucidated (Lurie and Watkins, 2012). To date, the most investigated and widely accepted hypothesis about the scald development in apple is related to the accumulation of products derived by the autoxidation of α-farnesene, such as conjugated trienols (Whitaker et al., 1997, 2000; Rowan et al., 2001) or 6-methyl-5-hepten-2-one (MHO; Mir et al., 1999; Wang and Dilley, 2000; Rudell et al., 2009; Farneti et al., 2014; Busatto et al., 2014). This reaction, together with the oxidation of polyphenol compounds can be ascribed as the responsible mechanism or the brown coloration occurring with the onset of the superficial scald (Du and Bramlage, 1995; Abbasi et al., 2008; Busatto et al., 2014).

The occurrence of scald seems to be cultivar specific, with some varieties, such as 'Cripps Pink', being more resistant than others such as 'Granny Smith', 'Red Delicious', and 'Fuji', which appear to be predisposed to the onset of the disorder (Whitaker et al., 2000; Tsantili et al., 2007). Maturity stage at harvest is the other major factors influencing fruit susceptibility to scald, with more mature fruits tending to have reduced scald severity than immature fruits (Wang and Dilley, 1999; Whitaker et al., 1997; Watkins et al., 2000; Lurie and Watkins, 2012).

To date, several methods for the prevention of this phenomenon have been already implemented, such as low-oxygen controlled atmosphere storage, forced ventilation, heat shock and diphenylamine (DPA) treatment (Lurie and Watkins, 2012). However the DPA application is nowadays under a sever review, due to possible risks associated with the employment of this compound (Song et al., 2014). As alternative molecule, the postharvest management took advantage of 1-MCP, an ethylene inhibitor widely adopted to extend storage capacity of climacteric fleshy fruits (Watkins, 2006). Applications of 1-MCP successfully reduce scald incidence, even in susceptible cultivars (Tsantili et al., 2007). However, 1-MCP efficacy resulted to be strongly affected by maturity stage and uniformity of fruit batch (Jung and Watkins, 2008; Whitaker et al., 1997; Busatto et al., 2014).

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Consequently, a decision support-tool that can identify the fruit maturity at harvest is needed. This decision support-tool would enable the division of fruits into homogenous maturity batches which can be subjected to differential post-harvest management regimes. In response to this need, extensive research has been focused on the development of non-destructive vis/NIR technology for assessing fruit maturity stage (Bobelyn et al., 2010; Nicolaï et al., 2007). Recently research shows the correlation between the index of absorbance difference (I_{AD}), assessed by using VIS technology, and ripening related attributes in apple; these results show the possible potential for practical implementation in commercial lines (Nyasordzi et al., 2013). The implementation of I_{AD} in storage, packlines and distribution would provide a significant decision support tool in post-harvest management.

The scope of this study was to investigate the use of I_{AD} as a possible decision-support system to tailor 1-MCP application in order to maximize its efficacy in scald control.

2. Materials and methods

2.1. Plant material and growing conditions

'Granny Smith' and 'Cripps Pink' apple were selected for this study, with the former being highly susceptible to scald, while the latter more resistant. Apple trees were grafted on M.9 rootstock. All trees were 4-year old and were planted at 3.8 m × 0.8 m. The commercial orchard used for the experiments was located in Bagnacavallo (RA), Northern Italy. Standard cultural and disease management strategies were applied.

Both cultivars were harvested at the commercial harvest date assessed by starch content and colouration parameters for three consecutive seasons (2010, 2011, and 2012).

2.2. Maturity assessment

For each cultivar, maturity at harvest was determined using the "index of absorbance difference" (I_{AD}) assessed by the DA-Meter which is a portable and non-destructive device based on visible/Near Infra Red (vis/NIR) spectroscopy (TR, Forlì, Italy) (Ziosi et al., 2008). I_{AD} values usually range from 2.2 to 0 where the higher number indicates a less ripe fruit characterized by a greater amount of chlorophyll present in the apple. Maturity stage was assessed according to methods reported by Ziosi et al. (2008) and Nyasordzi et al. (2013). At harvest two maturity classes were identified for 'Granny Smith' (I_{AD} classes: "2.0–1.8" and "1.8–1.6") and three maturity classes for 'Cripps Pink' (I_{AD} classes: "1.2–1.0", "1.0–0.8" and "0.8–0.6").

In each maturity class, for each cultivar, 360 apples were selected for post-harvest storage and 1-MCP treatment.

2.3. 1-MCP application

1-MCP was applied at harvest to 180 fruit for each I_{AD} class. 1-MCP treatment was carried out as SmartFresh^{TM} (0.14% active ingredient) according to manufacturer's instructions (AgroFresh, Rohm and Haas, Philadelphia, Pennsylvania, USA), reaching a final gas concentration of 0.7 $\mu l\,l^{-1}$. Fruit were exposed to 1-MCP for 24 h at 20 °C. After exposure, fruit boxes were ventilated and placed in cold storage at +1 °C for 6 months in commercial storage conditions. The second batch of 180 non-treated fruits was used as control and processed in the same way of the 1-MCP treated ones.

2.4. Superficial scald incidence evaluation

50 fruit per treatment for each I_{AD} class were removed from cold storage every two months and kept at 20 °C for 7 days. After this period, scald incidence was visually assessed and recorded as percentage of affected fruit.

2.5. Extraction and quantification of α-farnesene, CTols and ethylene emission

At harvest and at two month intervals during storage, peel tissue, including the epidermis and 1–2 mm of hypodermal cortex, was excised from the equatorial region of 5 fruits per treatment from each maturity class and cultivar and immediately frozen in liquid N_2. Pooled samples of about 20–30 g were stored at −80 °C in sealed bags. Extraction of α-farnesene and CTols was performed in three replicates for each pooled sample; extracts were analyzed by a HPLC equipped with a Photodiode Array Detector (Waters 2996), fitted with a 4.6 × 250 mm, Luna C18 column (Phenomenex, Torrence, CA), in accordance with the methodology reported by Whitaker et al. (2000). The α-farnesene and CTols identification was carried out through comparison of the retention time values and UV spectra with authentic standards (detected between 210 and 400 nm wavelength). For each compounds, the concentrations, expressed in $\mu g\,g^{-1}$ fresh weight (FW), was calculated from curves obtained with known amount of the corresponding external standard.

Ethylene production was measured by placing the whole fruit in a 1 l glass jar sealed with an air-tight lid equipped with a rubber stopper, and left at room temperature for 1 h. An aliquot of 10 ml of the headspace was collected and injected into a Dani HT 86.01 packed-gas chromatograph (Dani, Milan, Italy) as described by Bregoli et al. (2002).

3. Results

3.1. Evaluation of apple ripening based on I_{AD}

The index of absorbance difference (I_{AD}) assessed on 'Granny Smith' and 'Cripps Pink' decreased in the last 40 days before harvest in accordance with the enhanced ripening conditions (Supplementary Fig. 1). The I_{AD} evolution between the 150 and 190 days after the full bloom (DAFB), in fact, showed an average decrease from 2.0 to 1.75 for 'Granny Smith' and from 1.95 to 1.10 for 'Cripps Pink' (Supplementary Fig. 1). During the three experimental seasons both cultivars revealed a broad fruit ripening distribution based on I_{AD} values at harvest (190 DAFB) (Fig. 1A and B). In detail, 'Granny Smith' apples were ranked mainly in four ripening classes between I_{AD} values of 2.0 and 1.6 (95% of the fruit), while for 'Cripps Pink' the distribution was more wide with the 95% of the fruit ranked between 1.5 and 0.5 (Fig. 1A and B). In this study the ripening classes of 'Granny Smith' and 'Cripps Pink' were merged in two ("2.0–1.8" and "1.8–1.6") and three ("1.2–1.0", "1.0–0.8", and "0.8–0.6") sub-classes, respectively.

I_{AD} differences assessed at harvest were significantly linked with the main ripening/quality traits of apple, namely ethylene, firmness, total soluble solids, and starch content (Table 1). However, despite the high correspondence between ethylene content and I_{AD} discovered in 'Cripps Pink' (R^2 = 0.93), the correlation between I_{AD} and quality traits was not as clear as in 'Granny Smith' apples especially for firmness ('Granny Smith', R^2 = 0.86; 'Cripps Pink', R^2 = 0.55) and starch levels ('Granny Smith', R^2 = 0.82; 'Cripps Pink', R^2 = 0.61).

1-MCP treatment strongly reduced the 'Granny Smith' and 'Cripps Pink' fruit ethylene emission during the six months of storage as compared to control. No differences were observed in 1-MCP treated fruit between the maturity classes based on I_{AD} values (Fig. 2A and B). In control fruit, ethylene emission significantly increased already after two months of storage without any relevant differences related to the harvest ripening stage. Any season's