Acetaldehyde and Ethanol Metabolism during Conditioning and Air Storage of ‘Honeycrisp’ Apples

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Abstract. ‘Honeycrisp’ apples are susceptible to the physiological disorder soft scald, especially when stored at temperatures close to 0 °C. The disorder can be reduced by a conditioning treatment of 10 °C for 7 days before storage, but little is known about the underlying physiology of disorder development. The effects of storing ‘Honeycrisp’ apples in air at 0.5 °C for a total of 140 days, without and with conditioning, on internal ethylene concentration (IEC), ethanol and acetaldehyde concentrations, and activities of alcohol dehydrogenase (ADH) and pyruvate decarboxylase (PDC) were investigated in relation to soft scald incidence. Fruit were selected on the basis of background color (chlorophyll concentration) using a nondestructive delta absorbance (DA) meter to minimize variability of fruit maturation. Conditioning reduced soft scald incidence to 1% compared with 28% in unconditioned fruit. During the conditioning period, IECs were usually greatest in the conditioned fruit, with no effect on ethanol and acetaldehyde concentrations. During subsequent storage, IEC was greatest in conditioned fruit, whereas ethanol and acetaldehyde concentrations were generally less. However, ADH and PDC activities were unaffected consistently by conditioning or during storage.

‘Honeycrisp’ apples can be highly susceptible to the development of low temperature storage disorders such as soft scald (an external injury) and soggy breakdown (an internal injury) (Lachapelle et al., 2013, 2017; Tong et al., 2003; Wargo and Watkins, 2004; Watkins et al., 2004, 2005). Fruit can develop either one or both disorders, and they apparently represent different expressions of low temperature injury. Also, soggy breakdown may be a variant of low temperature breakdown, the more common low temperature disorder (Wills and Scott, 1970). In Nova Scotia, low temperature injury in ‘Honeycrisp’ is predominantly expressed as low temperature breakdown (DeLong et al., 2006). Regardless of expression type, incidence typically occurs within the first 6 to 8 weeks of storage and is not progressive after removal to warm temperatures. More mature fruit have greater susceptibility to disorder development, although effects of harvest date can be inconsistent (Moran et al., 2010; Tong et al., 2003; Watkins et al., 2005). Recently, a new indicator of harvest maturity, the delta absorbance (DA) meter, which assesses changes in background skin color (chlorophyll concentration) of the fruit, has been developed and tested on several cultivars, including ‘Empire’, ‘Granny Smith’, ‘Golden Delicious’, ‘Pink Lady’, and ‘Red Delicious’ (Cocetta et al., 2017; Doerflinger et al., 2016; Nyasordzi et al., 2013). Low readings, which indicate a more yellow background color (low chlorophyll concentrations) and advanced maturity are associated with high soft scald susceptibility in ‘Honeycrisp’ apples (unpublished data).

An obvious strategy to avoid low temperature storage disorders is to increase storage temperatures, but soggy breakdown and soft scald can develop even at 2 to 3 °C in late-harvested fruit (Moran et al., 2010; Watkins et al., 2004, 2005). The discovery that soft scald development in ‘Honeycrisp’ apples can be controlled by a conditioning period of 7 d at 10 °C (Watts and Rosenberger, 2000) was confirmed by subsequent research (DeLong et al., 2004, 2009; Moran et al., 2010; Watkins et al., 2004), although control may not always be complete, especially in more mature fruit stored at temperatures less than 3 °C (Moran et al., 2010; Watkins et al., 2004). Conditioning has little effect on quality factors such as flesh firmness, titratable acidity (TA), and soluble solids concentrations (SSC), although bitter pit incidence and skin greasiness may increase (DeLong et al., 2006, 2009; Watkins et al., 2004). Conditioning is widely used by commercial ‘Honeycrisp’ storage operators to reduce risk of disorder development.

The etiology of soft scald development is not well understood, nor is the mechanism by which conditioning can decrease the disorder. Soft scald incidence is related to oxidation of unsaturated fatty acids in the surface lipids and elevated hexanol concentrations in fruit (Hopkirk and Wills, 1981). Untargeted metabolic profiling of ‘Honeycrisp’ apples with varying degrees of susceptibility to soft scald revealed changes in a number of distinct pathways preceding and concurrent with disorder development, including elevated γ-aminobutyric acid (GABA), 1-hexanol, acylated steryl glycosides, and free rho-coumaryl acyl esters (Leisso et al., 2013, 2015, 2016). Tissues of fruit with soggy breakdown have greater concentrations of GABA, glycerol, sitosteryl (6-O-palmitoyl)-d-gluco-side and sitosteryl (6-O-stearate)-d-gluco-side, and triacylglycerides containing combinations of 16:0, 18:3, 18:2, and 18:1 fatty acids than fruit without injury (Leisso et al., 2013, 2015, 2016). A proteomic study on ‘Ambrosia’, an apple cultivar also susceptible to soft scald, revealed metabolic differences in fruit that were conditioned compared with unconditioned fruit (Luo et al., 2018). However, the reduction of soft scald incidence with conditioning was trivial, and therefore it is uncertain that metabolic differences are really meaningful in relation to disorder incidence.

At harvest and under aerobic storage conditions, apples usually have low acetaldehyde and ethanol concentrations, as well as low pyruvate decarboxylase (PDC) and alcohol dehydrogenase (ADH) activities (Pesis, 2005). Both compounds are precursors of aromatic volatiles in apple fruit, with the activity of alcohol acyltransferase being the last step of volatile compound synthesis to produce esters from alcohols (Espino-Diaz et al., 2016). Ethanol can accumulate in apples if left on the tree and harvested late (Nichols and Patterson, 1987), but most focus on the fermentation pathway, which is centered on anaerobic metabolism during the postharvest period (Pesis, 2005). Accumulation of acetaldehyde and ethanol is associated with stress, typically low oxygen and/or high carbon dioxide concentrations in the storage atmosphere (Saquet and Streif, 2008) or when skin coatings are applied (Bai et al., 2003). However, acetaldehyde and ethanol accumulations have been associated with physiologic disorders, including low temperature injuries and soft scald (Lyons, 1973; Miller, 1936; Thomas, 1931), although it is unclear whether acetaldehyde is a cause or a result of disorder development (Smagula and Bramlage, 1977).
Watkins et al. (2004) found that ethanol concentrations in cortical tissues of ‘Honeycrisp’ apples were greater in those stored at 0.5 °C than at 2.8 °C, and lowest in conditioned fruit after storage at either temperature. Although Leisso et al. (2015) found high acetaldehyde and ethanol concentrations in peel tissues of ‘Honeycrisp’ fruit with soft scald (Leisso et al., 2016). In contrast, we have consistently found high acetaldehyde and ethanol concentrations in peel tissues of ‘Honeycrisp’ fruit with soft scald (unpublished data).

The primary objective of this study was to compare acetaldehyde and ethanol concentrations, and activities of PDC and ADH, with soft scald development in ‘Honeycrisp’ fruit stored at 0.5 °C with and without conditioning. We used the DA meter to select fruit with greater risk of disorder susceptibility and to minimize variation of fruit maturity within the experimental treatments.

Materials and Methods

Plant material. The fruit from nine ‘Honeycrisp’ apple (Malus domestica Borkh.) trees (16 years old, grafted on M.9 rootstock, at the Cornell Orchard, Ithaca, NY) were strip-picked on 20 Sept. 2015. Uniform size and unblemished fruit were then sorted in the laboratory using a DA meter (TR Turoni srl, Forli, Italy). Each fruit was measured on the unblushed and blushed side, and then separated into DA categories. A total of 568 fruit from DA meter category 0.2 to 0.4 were randomly allocated into six replicates. Three replicates were stored at 0.5 °C immediately, and three replicates were conditioned in air at 0.5 °C for 7 d and then stored at 0.5 °C.

Fruit sampling. Three replicates of five fruit were used at harvest for initial assessments. The internal ethylene concentration (IEC) of each fruit was measured for 1 mL gas samples taken from the core of each apple and injected into a Hewlett-Packard 5890 series II gas chromatograph (Hewlett-Packard, Wilmington, DE), as described by Watkins et al. (2000). Firmness was measured on opposite peeled sides of each fruit using a Guss Fruit Texture Analyzer [Guss Manufacturing (Pty) Ltd., Strand, South Africa] fitted with an 11.1-mm-diameter probe, and the expressed juice was used for measurement of the SSC with a PR-100 refractometer (Atago Co. Ltd., Tokyo, Japan). TA was measured by titrating the juice to pH 8.1 with 0.1 m NaOH (Mettler DL 12 Titrator, Highstown, NJ). The starch pattern index (SPI) of each fruit cut at the equator was measured using the Cornell generic chart in which 1 represents 100% stained starch and 8 represents 0% stained starch (Blanpied and Silsby, 1992).

Five fruit were sampled from each replicate at 0, 2, 5, 7, 8, 14, 21, 35, 56, 77, 105, and 140 d (during conditioning and during air storage at 0.5 °C). Each replicate was removed from cold storage immediately before measurement of IEC and peeling, and was processed rapidly to ensure that warming of fruit was minimized. Each fruit was peeled by taking skin from the stem to the calyx, and was immediately dropped into liquid nitrogen. Samples were stored at −80 °C until they were ground in liquid nitrogen using an A11 analytical grinding mill (IKA Works, Wilmington, NC) (Lee et al., 2012). The fine powder was stored at −80 °C.

Acetaldehyde and ethanol. Five grams of powder was added to 2.5 g saturated NaCl in 20-mL vials fitted with septa, and were kept at −20 °C until further use. The vials were heated in a dry bath incubator (Fisher Scientific Co., Waltham, MA) at 80 °C for 20 minutes. A headspace sample of 0.5 mL was analyzed using a gas chromatograph (Agilent 6850, Agilent, Santa Clara, CA) with Agilent ChemStation version B.04.01 software on a Hewlett Packard Compaq computer. A 15-m × 0.53-mm-i.d. Restek wide-bore capillary column coated with 1.0 µm Stabilwax (Restek Corp., Bellefonte, PA) was used. The carrier gas was high-purity helium at 6 mL·min⁻¹, with hydrogen at 40 mL·min⁻¹ and compressed air at 400 mL·min⁻¹. The inlet and detector temperatures were 220 °C and 245 °C, respectively. The temperature program was held at 40 °C for 4 min, increased to 200 °C at 30 °C·min⁻¹, and was held for 5 min before cooling to 40 °C as described by Fernandez-Trujillo et al. (2001). Areas with identical retention times were compared with standard curves for ethanol and acetaldehyde. Concentrations are presented as milligrams per kilogram on a fresh weight basis.

ADH and PDC enzyme extraction and measurement. A total of 0.4 g frozen powder was extracted using 1 mL 85 mm 2-(N-morpholino)ethanesulfonic acid (MES) pH 6.0, 50 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 1% (w/v) polyvinylpyrrolidone, and 0.1% (v/v) Triton X-100. The mixture was homogenized using a vortex mixer and filtered with two layers of cheesecloth. The harvest index for the fruit in the 0.2- to 0.4-DA meter category was 8.9 ± 0.5 mm·L⁻¹, whereas that of fruit from each replicate, an average of 35 fruit, was assessed for presence or absence of soft scald and soggy breakdown, as well as any other disorders, as percentages, after 140 d ± 7 d at 20 °C. Each fruit was cut four to five times from the calyx to the stem.

Protein concentrations were measured according to the Bradford (1976) method, using a bovine serum albumin standard curve.

Physiologic disorders. The remaining fruit from each replicate, an average of 35 fruit, were stored at −80 °C and 0 °C for 2, 5, 7, 14, 21, 35, 56, 77, and 105 d, and then analyzed for physiological disorders. The ADH activity was measured according to Kagi and Vallee (1960) by using 1 mL 50 mM sodium pyrophosphate buffer, pH 8.5, 100 µL 75 mM ethanol, 1.5 mL 5 mM NAD, and 100 µL of the enzyme extract. Increasing absorbance at 340 nm was recorded up to 6 min at 25 °C. One unit converted 1 µmol ethanol to acetaldehyde per minute, and activity is expressed as unit per milligram protein.

The harvest indices for the fruit in the 0.2- to 0.4-DA meter category were 8.9 µL·L⁻¹ IEC, 58 N flesh firmness, 12.8% SSC, and 0.55% TA, with an SPI of 8.

The IEC of fruit placed immediately into cold storage decreased rapidly, but then increased to 3.8 µL·L⁻¹, whereas that of fruit kept at 10 °C for 7 d increased to 14.3 µL·L⁻¹ (Fig. 1A). After 24 h of cold storage, the IEC decreased rapidly to 2.3 µL·L⁻¹, whereas that of fruit kept at 0 °C for 10 d increased to 13.5 µL·L⁻¹ (Fig. 1B).

Results

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Fig. 1. Internal ethylene concentration (IEC) in ‘Honeycrisp’ apples stored at 0.5 °C (no conditioning) or 10 °C for 7 d (conditioning) (A), and then stored at 0.5 °C for up to 140 d (B). Data are presented as means ± se (n = 5).

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of fruit from untreated and conditioning treatments were both 5.8 \mu L^{-1} (Fig. 1B). The IEC then fluctuated during cold storage, with the IEC of the conditioned fruit usually greater than that of the unconditioned fruit (Fig. 1B). Over all sampling times, the IEC of fruit during conditioning was 11.4 \mu L^{-1} compared with 4.2 \mu L^{-1} in unconditioned fruit \((P < 0.001)\), and during storage was 8.3 \mu L^{-1} in conditioned fruit compared with 6.0 \mu L^{-1} in unconditioned fruit \((P < 0.001)\).

Acetaldehyde concentrations in fruit stayed stable during the conditioning period. Over all sampling times, concentrations were unaffected by treatment, although they tended to be slightly greater in fruit placed directly in storage at 0.5 \degree C (Fig. 2A). Ethanol concentrations were unaffected by conditioning (Fig. 2C). Both acetaldehyde and ethanol concentrations fluctuated during subsequent cold storage, but were generally greater in fruit placed directly in storage at 0.5 \degree C than in conditioned fruit (Fig. 2B and D). Over all sampling times, acetaldehyde concentrations during storage were 1.9 mg kg^{-1} in conditioned fruit compared with 3.5 mg kg^{-1} in unconditioned fruit \((P < 0.01)\), and for ethanol were 25 mg kg^{-1} in conditioned fruit compared with 46 mg kg^{-1} in unconditioned fruit \((P < 0.01)\). No consistent effects of conditioning treatment on PDC or ADH activity were detected, either during the conditioning period or during cold storage (Fig. 3A–D).

At the final sampling time of 140 d, soft scald incidence of fruit kept at 0.5 \degree C was 28% compared with 1% in the conditioned fruit. No soggy breakdown was detected. Low incidences of bitter pit, senescent breakdown, and wrinkly skin were observed in fruit of both treatments, but were not statistically different (data not shown).

**Discussion**

Most recommendations for postharvest handling of apple fruit have focused on rapid cooling to reduce ripening and senescence processes (Watkins, 2017). However, step-wise cooling has been recommended for control of core browning in ‘Granny Smith’, but with some loss of fruit condition (Little and Holmes, 2000). Similarly, a storage regimen of 10 \degree C for 10 d, 4 \degree C for the next 20 d, and 0 \degree C for the remaining 150 d resulted in the least superficial scald incidence in fruit without 1-methylcyclopropene treatment (Moggia et al., 2009). We found that fruit with conditioning had lower soft scald incidence than unconditioned fruit. Soft scald and soggy breakdown are thought to be low temperature/chilling injuries (CIs) (Pierson et al., 1971; Smock, 1977), as is superficial scald (Watkins et al., 1995). The effects of delayed cooling, step-down cooling, or conditioning periods are therefore consistent with similar effects on reducing CI incidence of other fruit types; these are used commercially when effects on fruit quality are not negative. The biochemical changes underlying the effects of conditioning on disorders are not understood, but appear to involve a complex interaction between the effects of maturity and ripening on postharvest changes. For stone fruit, for example, advancing ripening processes appear to be associated with lower CI incidence (Lurie and Crisosto, 2005). In the current study, fruit had greater IECs during conditioning than without conditioning, and these greater IECs were found during the remainder of time in storage, suggesting that a greater rate of metabolism was induced by conditioning.
Conditioned fruit also had lesser ethanol and acetaldehyde accumulations than those found in unconditioned fruit during the cold storage period, but had no consistent effects on PDC and ADH activities. The relationships between fermentation products, especially acetaldehyde, and storage disorders has been the subject of debate (Smagula and Bramlage, 1977). Acetaldehyde is regarded as a more toxic compound than ethanol (Chervin et al., 1999). A feature of soggy breakdown and soft scald has been acetaldehyde and ethanol accumulations (Miller, 1936; Smagula and Bramlage, 1977; Thomas, 1931), and ethanol accumulation is associated with incidence of both disorders in ‘Honeycrisp’ apples (Watts et al., 2004). Although Leissio et al. (2016, 2017) found that ethanol accumulation was more consistently associated with soggy breakdown than with soft scald, we have consistently observed accumulations of ethanol in peel tissues of fruit with soft scald (Al Shoffe et al., 2017). Conditioning reduces soft scald incidence in fruit, but the mechanism by which conditioning inhibits development of the disorder is not known.

The absence of relationships between acetaldehyde and ethanol accumulation and associated enzyme activities is not uncommon. Examples include kiwifruit and pear, in which ADH activity was less during storage despite an increase in ethanol accumulation (Botondi et al., 2012; Lara et al., 2003). Even less ADH activity may be enough for ethanol production (Chervin et al., 1999; Echeverria et al., 2004; Prestage et al., 1999).

In conclusion, the reduction of soft scald incidence by conditioning supports the view that the disorder is a CI. The effects of conditioning were associated with greater IECs and presumably faster metabolism. Conditioning resulted in lesser acetaldehyde and ethanol concentrations in ‘Honeycrisp’, but no consistent effects on ADH and PDC activities, during storage. More research to understand the metabolic effects of conditioning on fruit is needed.

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