

Fruit Maturity Based on I_{AD} Values in Relation to Preharvest 1-Methylcyclopropene Treatment and Postharvest Physiological Disorder Development in ‘Honeycrisp’ Apples

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Keywords. *Malus domestica* Borkh, carotenoids, chlorophyll, fruit maturity, sugars, volatiles

Abstract. ‘Honeycrisp’ apples are prone to physiological disorder development during storage, fruit susceptibility to disorder incidence being affected by harvest date. The effects of fruit maturity from untreated trees and those sprayed at 1 week before the first harvest with 1-methylcyclopropene (1-MCP; Harvista™) were investigated by categorizing fruit into index of absorbance difference (I_{AD}) values before storage. Maturity indices, chlorophyll, carotenoid, and sugar concentrations were assessed at harvest. The incidences of physiological disorders were assessed after 4 months plus 7 days at 20 °C. The internal ethylene concentration and the starch pattern index were lower in fruit treated with preharvest 1-MCP compared with untreated fruit, while fruit firmness was higher in preharvest 1-MCP-treated fruit. The difference was more pronounced in fruit with higher I_{AD} values (higher chlorophyll) categories at harvests 2 and 3. Chlorophyll a and total chlorophyll concentrations were positively correlated with the I_{AD} values in both untreated and preharvest 1-MCP-treated fruit. Acetaldehyde and ethanol were unaffected by harvest time, but preharvest 1-MCP-treated fruit had lower ethyl acetate accumulation at all harvests. Sucrose, fructose, glucose, galactose, sorbitol, and malic acid concentrations were often higher in preharvest 1-MCP-treated fruit than in untreated fruit. Soft scald incidence was higher in fruit with lower I_{AD} values. However, the disorder was lowest in harvest 1 and 2 fruit of higher I_{AD} values when the fruit were treated with preharvest 1-MCP. Additionally, fruit senescence was higher in late-harvested fruit. Principal components analysis, multivariate analysis, and the nonlinear iterative partial least square algorithm showed that fruit physiological disorder development after storage was correlated with fruit maturity based on the I_{AD} value and maturity at harvest as affected by preharvest 1-MCP treatment.

Fruit maturity and harvest time play a crucial role in the occurrence of physiological disorders of ‘Honeycrisp’ apples. Early harvests are associated with susceptibility of fruit to bitter pit (Al Shoffe et al. 2020; DeLong et al. 2014; Öz et al. 2025), while late harvests are associated with soft scald, soggy breakdown, senescent breakdown, and flesh browning (Al Shoffe et al. 2020, 2023; Moran

et al. 2010; Öz et al. 2025; Prange et al. 2011; Tong et al. 2003; Watkins et al. 2005). Soft scald is a chilling injury characterized by distinct, irregularly shaped brown patches on the fruit’s peel. The mechanisms driving soft scald development remain poorly understood. Soft scald has been associated with the oxidation of unsaturated fatty acids in surface lipids and elevated hexanol levels (Hopkirk and Wills

1981). The disorder is a stress response related to glutamate and phenolic metabolism as well as lipoxygenase and C6 volatile metabolism (Leisso et al. 2016).

The Delta Absorbance (DA) meter, a non-destructive harvest index, is widely used to assess chlorophyll concentrations (specifically chlorophyll a) in fruit skin. The DA meter measures the index of absorbance difference (I_{AD}), calculated as the absorbance difference between 670 and 720 nm ($A_{670} - A_{720}$). Higher I_{AD} values indicate greater chlorophyll concentrations. Relationships between I_{AD} values and other harvest indices, as well as the predictive utility of I_{AD} values for storage quality of apple fruit, have been investigated (Costamagna et al. 2013; Doerflinger et al. 2024, 2019; DeLong et al. 2014; Moran et al. 2020; Mostofi and DeEll 2024; Nyasordzi et al. 2013). Changes of maturity indicated by I_{AD} values have been linked to fruit susceptibility to physiological disorders, including bitter pit, senescent breakdown, soft scald, superficial scald, stem end flesh browning, and watercore (Doerflinger et al. 2024, 2015; DeLong et al. 2014; Farneti et al. 2015; Knutsen et al. 2015; Lee et al. 2019; Moran et al. 2020).

A preharvest formulation of the ethylene inhibitor 1-methylcyclopropene (1-MCP), commercially available under the trade name Harvista™, is registered as a plant growth regulator (PGR) to delay fruit drop by affecting maturation and ripening (Al Shoffe et al. 2021; Byers 1997; DeEll and Ehsani-Moghaddam 2010; Doerflinger et al. 2024, 2016, 2019; Johnson and Farcuh 2024; Sakaldas and Gundogdu 2016; Varanasi et al. 2013; Watkins et al. 2010; Yoo et al. 2015; Yuan and Li 2008). Preharvest 1-MCP treatments also reduce the incidence of physiological disorders such as superficial scald, soft scald, and internal browning (Al Shoffe et al. 2021; DeEll and Ehsani-Moghaddam 2010; Doerflinger et al. 2017; McArtney et al. 2008). However, it has also been associated with an increased risk of bitter pit and external CO₂ injury (Al Shoffe et al. 2021; DeEll and Ehsani-Moghaddam 2012).

As apple fruit matures, the balance between sweetness and acidity undergoes changes that influence the sensory quality of apples. The primary sugars in apples, fructose, sucrose, and glucose, vary in concentration depending on the cultivar, growing region, and weather conditions during fruit development (Musacchi and Serra 2018). While fructose accumulation stops at 5 weeks before fruit harvest, sucrose continues to accumulate to harvest in ‘Honeycrisp’ (Zhang et al. 2010). Although sorbitol is present in smaller quantities in the fruit compared with the leaves, it plays a crucial role in sugar metabolism and accumulation during fruit development. During apple fruit development, sorbitol and sucrose produced through photosynthesis are translocated from the leaves (source) to the fruit (sink) (Berüter 1985; Li et al. 2018). The degradation of starch at the time of maturity onset in the later stages of fruit development contributes to an increase in the fruit soluble solids content up to the point

of harvest (Li et al. 2016; Zhang et al. 2010). Fruit ripening is often associated with the breakdown and solubilization of pectic polysaccharides in the primary cell wall, along with a reduction in galactose content from the side chains of these polymers (Redgwell et al. 1997). Primary and secondary metabolic processes are associated with changes in pigment composition (Li et al. 2013). Color changes of the skin are also associated with changes in chlorophylls and carotenoids (Solovchenko et al. 2005).

At harvest and under aerobic storage conditions, apples usually have low acetaldehyde and ethanol concentrations, as well as low pyruvate decarboxylase and alcohol dehydrogenase activities (Pesis 2005). Ethanol can accumulate in apples if left on the tree and harvested late (Nichols and Patterson 1987). However, acetaldehyde and ethanol accumulations have been associated with higher incidence with low-temperature injuries such as soft scald (Al Shoffe et al. 2018). The objective of this study was to examine the effects of preharvest 1-MCP application on I_{AD} values, fruit maturity, and quality in relation to the incidence of physiological disorders in 'Honeycrisp' apples during storage.

Materials and Methods

Plant material. 'Honeycrisp' apple (*Malus domestica* L. Borkh) 13-year-old trees grafted on M.9 rootstock were grown at the Cornell Orchards in Ithaca, NY, USA. Nine replicates of three trees each were either untreated or sprayed with the preharvest formulation of 1-MCP (Harvista™) (AFxRD-038; AgroFresh Inc., Dow AgroSciences, Spring House, PA, USA) 1 week before the first harvest. Harvista™ was applied at the standard industry rate of 13.8 kg·ha⁻¹ (6.8 g·L⁻¹), mixed with 0.1% Silwet L-77 (Helena Chemical Company, Collierville, TN, USA), using a CO₂ pressurized backpack sprayer (Bellspray, Opelousas, LA, USA) fitted with a Tee Jet 8004VS flat fan nozzle (Spraying Systems, Wheaton, IL, USA).

All fruit from each of the three replicates of untreated and treated plots were harvested on 16 Sep 2013 (H1), 23 Sep 2013 (H2), and 30 Sep 2013 (H3). The fruit were then sorted into 5 to 6 I_{AD} categories at 0.2-unit intervals with a DA meter (TR Turoni srl, Forli, Italy). The average of the blushed and unblushed sides of each fruit was used. For each replicate, the number of fruit in each category was

counted. Where a minimum of 15 fruit was available, 5 fruit were taken for assessment of harvest indices, and the remaining fruit were stored in air at 0.5°C up to 4 months plus 7 d at 20°C.

Harvest indices. The internal ethylene concentration (IEC) was measured on gas samples taken from the core of each apple as previously described (Watkins et al. 2000). Firmness, soluble solids concentration (SSC), and the starch pattern index (SPI) were measured as described by (Al Shoffe et al. 2021). Before starch staining, the skin of each fruit at the equator was peeled directly into liquid nitrogen and kept at -80°C until used. In addition, segments from opposite sides of each fruit were frozen in liquid nitrogen and stored at -80°C until used. The frozen samples were ground in liquid nitrogen using an analytical grinding mill (IKA works, Wilmington, NC, USA), and the fine powder was kept at -80°C for subsequent determination of sugars and acids.

Chlorophyll and carotenoid assessment. One gram of frozen peel tissue powder was extracted in 5 mL of 80% (v/v) acetone. The mixture was vortexed (Vortex-Genie-2®, model G-S60; Scientific Industries, Inc., Bohemia, NY, USA), shaken (VWR® mini shaker; VWR International, Avantor, Radnor, PA, USA) for 20 min at the 850 g setting, and centrifuged (Allegra® 64R centrifuge; Beckman Coulter, Indianapolis, IN, USA) for 15 min at 12,000 g, and the supernatant was collected. The chlorophyll and carotenoid contents were assessed by measuring the absorbances at 663 and 646 nm and at 470 nm, respectively, according to Lichtenthaler and Wellburn (1983). Chlorophyll a, chlorophyll b, and total carotenoids were calculated as mg·kg⁻¹ on a fresh weight basis.

Sugar and acid measurement. Powdered cortical tissues (100 mg) were put in 2-mL screw cap tubes, extracted in 1.4 mL of 75% methanol (-20°C) for 2 min, and vortexed for 10 s. Then 100 µL of ribitol was added as an internal standard, and the tubes were vortexed again for 10 s. The samples were shaken 30 min at 70°C in a thermo mixer at 960 rpm and then centrifuged for 10 min at 11,000 g. The supernatant was transferred to Schott GL 14 screw thread tubes (1.5 × 12 mm). Then 750 µL of chloroform (-20°C) and 1500 µL double-distilled water (4°C) were added, and the vials were vortexed for 10 s, the tubes were centrifuged 15 min at 2200 g, and 50 µL from the upper (polar) phase was transferred to a 2-mL round-bottomed Eppendorf tube. The samples were dried in vacuo without heating. The tubes were filled with argon, capped, placed in plastic bags with silica beads, and frozen at -80°C. Metabolites were identified by comparing fragmentation patterns with those in a mass spectral library generated in the gas chromatography mass spectrometry system (7890A GC/5975C MS; Agilent, Santa Clara, CA, USA) with an electron ionization source. Injection of 1 µL of sample was performed at 230°C in splitless mode with helium carrier gas flow set to 1 mL·min⁻¹. Chromatography was performed using a DB-5MS capillary column (20 m × 0.18 mm ×

0.18 µL) with a 5-m Duraguard column in front. The temperature program started at 70°C for 2.471 min followed by a 10.119°C·min⁻¹ ramp to 330°C for 2.471 min. The system was then temperature-equilibrated at 70°C for 5 min before the next injection. Mass spectra were collected at 5.6 scans/s with an m/z 50 to 600 scanning range. The transfer line temperature and the ion source temperature were set to 250 and 230°C, respectively, as described previously (Zhang et al. 2010).

Acetaldehyde, ethanol, and ethyl acetate measurement. A 5 g of juice was taken from two opposite segments of blushed and unblushed sides of each five-fruit replicate using an electric fruit juicer (model 11 JE21(6001); Acme Kitchenettes Corp., MI, USA). The juice was put in 20-mL glass vials, and then 2.5 mL of saturated salt (NaCl) was added and made to volume (10 mL) with distilled water as described by (Fernández-Trujillo et al. 2001). The vials were stored at -20°C before use. For volatile measurement, the vials were incubated in a dry bath (Fisher Scientific Co., Waltham, MA, USA) at 80°C for 30 min. A 1-mL sample from the headspace was analyzed using a gas chromatograph (Hewlett-Packard, Wilmington, DE, USA). The oven, inlet, and detector temperatures were 100, 220, and 245°C, respectively. Areas with identical retention times were compared with standard curves for acetaldehyde, ethanol, and ethyl acetate. Means are expressed as mg·kg⁻¹ on a fresh weight basis.

Assessment of disorders. The presence of external disorders (visual physiological disorders on the fruit skin such as bitter pit and soft scald) was assessed, and then each fruit was cut at least five times from stem to calyx end to assess the presence of internal disorders including senescent breakdown. The incidence of each disorder was expressed as a percentage.

Statistical analysis. The Tukey honestly significant difference test, Student's t test, and least significant difference were used to compare means at the 5% significance level. Means are used to present data in figures. Principal component analysis (PCA) was used to visualize the effects of harvest date, preharvest 1-MCP treatments, and I_{AD} category on fruit quality and incidences of disorders after storage. The Eigenvectors, which is a special set of vectors with a linear system of equations, were used to show the correlation between PCx and variables. The nonlinear iterative partial least square (NIPLS) algorithm based on the variable importance plot (VIP) vs. coefficients, for which a value of 0.8 was considered to be a small VIP as described by Al Shoffe et al. (2024), was used to derive the correlation between soft scald and harvest indices in relation to harvest date. All statistics were carried out using the JMP statistical program (JMP Pro 17.INK; SAS Institute Inc., Cary, NC, USA). Percentage data were arcsine transformed for analysis and presented as back-transformed means.

Data availability. The data will be made available on request.

Received for publication 10 Apr 2025. Accepted for publication 5 May 2025.

Published online 27 Jun 2025.

This work was supported by the National Institute of Food and Agriculture, US Department of Agriculture, Multistate under 1001075, NE1836, Improving Quality and Reducing Losses in Specialty Fruit Crops through Storage Technologies.

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Results

Fruit distribution and maturity. The distribution of fruit in the I_{AD} categories from H1 peaked at 0.61 to 0.8 for untreated fruit and 0.81 to 1.0 for fruit treated with preharvest 1-MCP (Fig. 1A). At H2, the distribution of untreated fruit peaked at 0.21 to 0.4, while that of 1-MCP-treated fruit peaked at 0.41 to 0.6 (Fig. 1B). At H3, ~50% of the untreated fruit were in the 0 to 0.2 category, compared with only 25% of 1-MCP-treated fruit. Overall, the percentage of untreated fruit decreased sharply from 0 to 0.2 to 0.61 to 0.8 (Fig. 1C). Conversely, fruit treated with 1-MCP showed a consistent percentage across the 0 to 0.6 range, followed by a linear decline at higher I_{AD} values (Fig. 1C). However, significant differences were only observed for I_{AD} values across each harvest (Fig. 1).

The IEC of untreated fruit was higher at H2 than at H1 and H3, regardless of I_{AD} category (Table 1). At this harvest, the IEC of fruit treated with preharvest 1-MCP were lower than that of the untreated fruit, but no effect of 1-MCP was detected at the other harvests.

Flesh firmness decreased with later harvests (Table 1). However, no significant effect of 1-MCP treatment or I_{AD} category on flesh firmness was detected within each harvest time except for the second harvest, in which firmness was 65.1 and 63 N for the 1-MCP-treated and untreated fruit, respectively.

Overall, the SSC was higher in fruit from H2 (12.5%) than in H1 (12.3%) and H3 (12.0%). There was no effect of 1-MCP treatment on SSC at H2, but at H1, the P value (0.0559) for the difference between 1-MCP treatment and the untreated control was close to 0.05. At H3, the SSC was lower in treated fruit than untreated fruit at the 1.01 to 1.20 category and intermediate at the 0.81 to 1.00 category.

The SPI increased with later harvest date and generally with lower I_{AD} category. Treatment effects within each harvest were small (Table 1).

Skin pigments. Higher chlorophyll a, chlorophyll b, and total chlorophyll concentrations were associated with higher I_{AD} categories (Table 2). Overall, I_{AD} values decreased with later harvest times. 1-MCP-treated fruit had higher chlorophyll concentrations, being $3.7 \text{ mg}\cdot\text{kg}^{-1}$ for chlorophyll a and $1.7 \text{ mg}\cdot\text{kg}^{-1}$ for chlorophyll b, compared with 3 and $0.7 \text{ mg}\cdot\text{kg}^{-1}$, respectively, in untreated fruit, but there was no consistent effect of 1-MCP treatment within each I_{AD} category. The

carotenoid concentrations showed a similar trend as the chlorophyll content to the I_{AD} value, but they were unaffected by harvest time (Table 2).

Volatiles at harvest. Acetaldehyde concentrations were unaffected by harvest time. However, in 1-MCP-treated fruit, acetaldehyde concentration gradually increased with higher I_{AD} values, whereas untreated fruit exhibited an inconsistent trend (Fig. 2A). At H2, acetaldehyde concentrations generally decreased with increasing I_{AD} values in both treated and untreated fruit, except for untreated fruit in the 0.6 to 0.8 I_{AD} range, in which acetaldehyde slightly declined to $0.06 \text{ mg}\cdot\text{kg}^{-1}$ (Fig. 2B). By the third harvest, acetaldehyde concentration slightly decreased with increasing I_{AD} values in both treated and untreated fruit, with 1-MCP-treated fruit showing higher concentrations than untreated fruit (Fig. 2C). Ethanol concentrations were higher in 1-MCP-treated fruit compared with untreated fruit from the first harvest, with an increase in the 1.0 to 1.2 I_{AD} category (Fig. 2D). At H2, ethanol levels in 1-MCP-treated fruit decreased after the 0.6 to 0.8 I_{AD} range, while in untreated fruit, the decline was delayed until the 1.0 to 1.2 I_{AD} range, after which ethanol levels also decreased (Fig. 2E). At H3, ethanol peaked in the 0.4 to 0.8 I_{AD} range in fruit untreated with 1-MCP. In contrast, ethanol levels remained stable in 1-MCP-treated fruit across the 0.0 to 1.0 I_{AD} categories before declining at 1.0 to 1.2 (Fig. 2F). However, ethanol concentration increased with delayed harvest, and it was 1.4, 2.6, and $3.7 \text{ mg}\cdot\text{kg}^{-1}$ for H1, H2, and H3, respectively, regardless of 1-MCP treatment or I_{AD} category.

Ethyl acetate concentrations decreased over harvest time, being 1.2, 0.1, and $0.1 \text{ mg}\cdot\text{kg}^{-1}$ for H1, H2, and H3, respectively, regardless of 1-MCP treatment or I_{AD} value. 1-MCP-treated fruit had lower ethyl acetate accumulation at all harvests. In the first harvest, untreated fruit in the 0.4 to 1.2 I_{AD} range showed the highest ethyl acetate concentration with significant differences compared with 1-MCP-treated fruit and to those from untreated fruit at I_{AD} category of 1.2 to 1.4. At H2, differences between untreated and 1-MCP-treated fruit were observed only in the 0.8 to 1 I_{AD} range. Neither 1-MCP treatment nor I_{AD} value affected the ethyl acetate concentration at H3 (data not shown).

Sugars and acids. Sucrose, sorbitol, and malic acid concentrations were higher in the H2 fruit compared with other harvests,

regardless of the I_{AD} value and 1-MCP treatment. Fruit treated with preharvest 1-MCP had $7.4 \text{ g}\cdot\text{kg}^{-1}$ of malic acid, compared with $6.6 \text{ g}\cdot\text{kg}^{-1}$ in untreated fruit (as main effects), regardless of harvest time or I_{AD} value. However, 1-MCP had no effect on the concentrations of other sugars when it was not categorized by I_{AD} value (Table 3). The highest glucose content was found in 1-MCP-treated fruit at an I_{AD} value of 1.2 to 1.4 at H1 and H3, compared with other I_{AD} categories in treated and untreated fruit (Table 3). Fructose concentrations were highest in treated fruit with I_{AD} values of 0.6 to 0.8 and 1.2 to 1.4, compared with other I_{AD} categories. However, the fructose levels did not show a consistent pattern across all I_{AD} values over different harvests (Table 3).

Physiological disorders. Delaying the harvest did not affect the incidence of soft scald. However, fruit treated with 1-MCP had less soft scald compared with the control, regardless of the I_{AD} value and harvest time (Fig. 3). For H1 fruit, 1-MCP treatment reduced soft scald incidence to nearly zero at an I_{AD} value of 1 to 1.2. Both untreated and 1-MCP-treated fruit had higher soft scald incidence at lower I_{AD} values, but it was lower in the 1-MCP-treated fruit compared with untreated ones (Fig. 3A). In the second harvest, only fruit treated with 1-MCP at an I_{AD} value of 0.8 to 1 had lower soft scald incidences compared with lower categories. However, the I_{AD} value did not affect soft scald incidence in untreated fruit at H2 (Fig. 3B). At H3, 1-MCP treatment had no significant effect on soft scald compared with untreated fruit across different I_{AD} categories (Fig. 3C).

Bitter pit incidences were low and unaffected by either harvest time or the 1-MCP treatment (data not shown). However, senescent breakdown, although initially trivial, increased with later harvests, and it was 0.2, 0, and 3.5% for fruit from H1, H2, and H3, respectively. The 1-MCP treatment had no impact on the development of senescent breakdown compared with untreated fruit (data not shown).

Principal components analysis. The two principal components, PC1 and PC2, account for 43% of the variation (Fig. 4). There was a clear separation between the second and third harvests compared with the first harvest. Chlorophyll, ethyl acetate, acetaldehyde, and flesh firmness were segregated in fruit from the first harvest compared with the other harvests. Conversely, sugars, malic acid, ethanol,

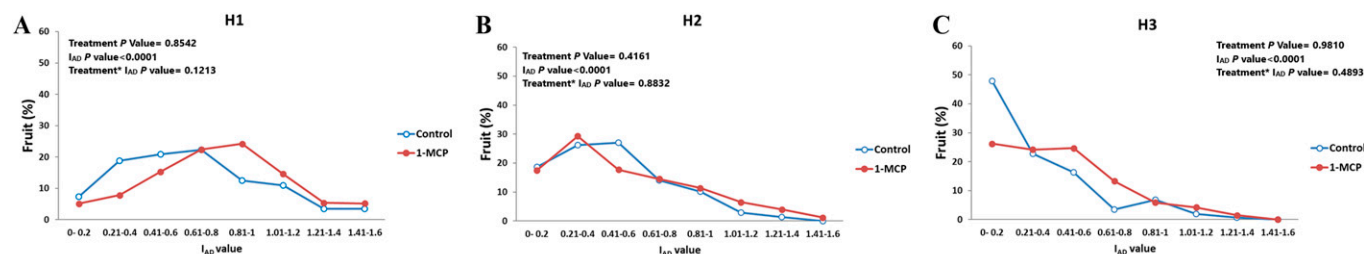


Fig. 1. Fruit distribution (%) per I_{AD} category of 'Honeycrisp' apples untreated (control) or treated with preharvest 1-methylcyclopropene (1-MCP) at harvest. Fruit were harvested at 1-week intervals; $n = 647, 693$, and 718 on 16 Sep 2013 (H1), 23 Sep 2013 (H2), and 30 Sep 2013 (H3), respectively.

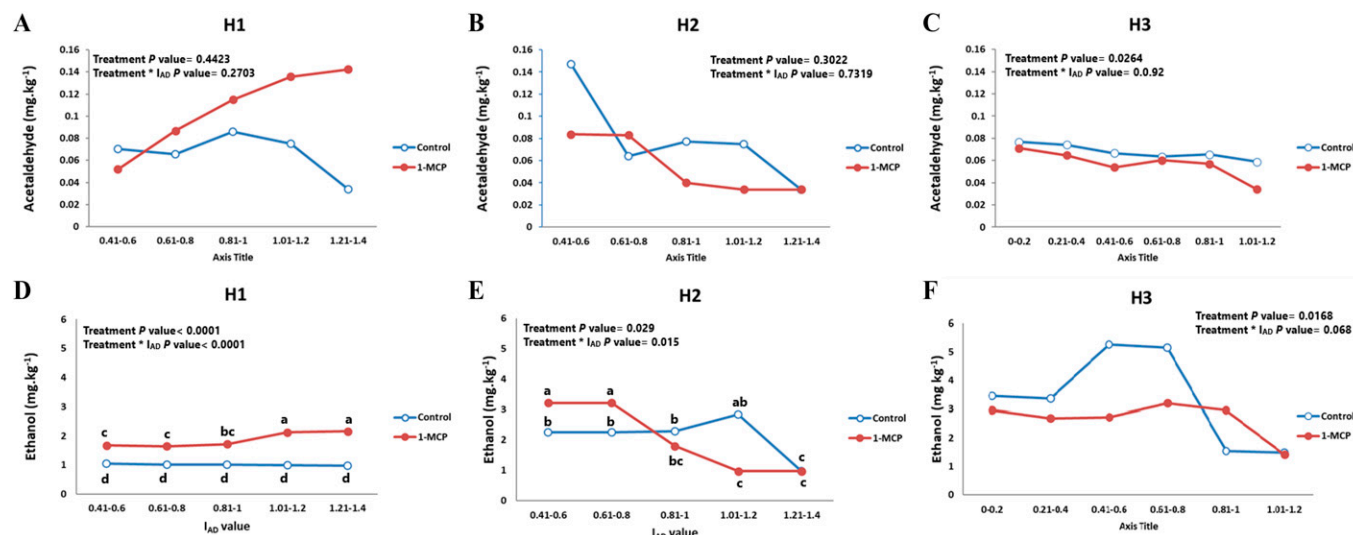


Fig. 2. Acetaldehyde (A–C), and ethanol (D–F) concentrations of ‘Honeycrisp’ apples categorized by I_{AD} value at harvest. Fruit were untreated (control) or treated with preharvest 1-methylcyclopropane (1-MCP) and harvested at three 1-week intervals. Different letters within the same figure indicate significantly different means ($P < 0.05$), $n = 3$.

‘Honeycrisp’ apples (Moran et al. 2020; Öz et al. 2025), and the current study extends the use of I_{AD} values to explore the effects of PGRs such as preharvest 1-MCP (Harvista™).

Preharvest 1-MCP sprays slightly delayed chlorophyll degradation and thus increased I_{AD} values of fruit compared with those that were untreated (Fig. 1). The application of preharvest 1-MCP and other plant growth regulators is time sensitive, depending on the maturity stage and the climacteric onset of the fruit (Al Shoffe et al. 2021). The limited influence of preharvest 1-MCP on chlorophyll concentrations and other fruit pigments in the final stages of fruit development may be attributed to its physiological effects on

the ethylene pathway, specifically by blocking ethylene receptors in the final stage of ethylene action and synthesis (Gorfer et al. 2022). In this context, we found that only fruit from H2 had lower IECs in treated compared with untreated fruit. However, 1-MCP inhibited IEC for 3 weeks postapplication. Fruit with high I_{AD} values showed lower IEC across all harvests (Table 1), consistent with the fact that less-mature apple fruit have lower ethylene production.

The effects of fruit maturity based on I_{AD} values on flesh firmness were variable, with 1-MCP-treated fruit showing higher firmness only in H2 (Table 1). Various studies have shown that changes in fruit firmness

in ‘Honeycrisp’ apples are minimal, likely due to low polygalacturonase activity (Harb et al. 2012). The SSC was only affected by delaying fruit harvest and varied between treatments and I_{AD} values (Table 1). The SPI (low numbers reflecting high starch concentrations) was negatively correlated with I_{AD} values. Previous studies have found that the SPI, often used as a primary maturity index, correlates highly with the I_{AD} in various apple cultivars (Moran et al. 2020; Sadar and Zannella 2019). However, prediction models of fruit maturity based on I_{AD} value are cultivar dependent (Sjöstrand et al. 2024) and vary by year (Mostofi and DeEll 2024).

The chlorophyll content in apple peel is associated with the I_{AD} index obtained from the DA meter in this study. The impact of 1-MCP treatment on maintaining higher chlorophyll levels compared with untreated fruit aligns with various studies showing that the degradation of chlorophyll pigments and the accumulation of carotenoids are correlated with ethylene biosynthesis. However, carotenoid levels did not increase with delaying harvest time in our study. This is likely because carotenoid accumulation occurs in early stages of fruit development (Ampomah-Dwamena et al. 2022).

Flavor development in apples occurs during ripening, with the highest concentration of endogenous volatiles at the climacteric peak (Dixon and Hewett 2000). Stress conditions such as hypoxia lead to high levels of acetaldehyde and ethanol, and upon returning to air, ethyl esters increase (Fellman et al. 2000), which are affected by pre- and post-harvest factors (Echeverría et al. 2004; Song and Bangerth 1996). In our study, acetaldehyde and ethanol, precursors of aroma volatiles in apple fruit, varied based on harvest time, preharvest treatment, and I_{AD} value (Fig. 2). At H1, the increase in fermentation products in fruit with higher I_{AD} values or in fruit treated with 1-MCP seems to correlate with low storage temperature stress.

Table 3. Glucose, fructose, and sucrose concentrations of ‘Honeycrisp’ apples categorized by I_{AD} value at harvest.

Harvest	I_{AD} value	Glucose (g.kg ⁻¹)		Fructose (g.kg ⁻¹)		Sucrose (g.kg ⁻¹)	
		Control	1-MCP	Control	1-MCP	Control	1-MCP
H1	0.41–0.60	3.2 b	3.1 b	65.7 ab	36.5 b	26.3 ab	18.5 b
	0.61–0.80	3.5 b	4.2 ab	70.5 ab	84.1 a	29.9 a	34 a
	0.81–1	3.7 b	4.4 ab	72.1 ab	83.1 ab	29.2 a	31.2 a
	1.01–1.2	3.8 b	4.7 ab	66.2 ab	82.0 ab	24.9 ab	28.4 ab
	1.21–1.4	4.7 ab	5.8 a	73.1 ab	87.8 a	24.5 ab	26.5 ab
LSD		1.95		47.8		10.63	
H2	0.41–0.6	4.5 a	4.8 a	91.7 a	91.1 a	43.3 a	41.2 a
	0.61–0.8	3.8 a	4.8 a	67.2 a	74.2 a	30.3 ab	32.8 ab
	0.81–1	4.0 a	5.2 a	66.6 a	92.5 a	27.4 ab	39.3 a
	1.01–1.2	4.0 a	4.6 a	75.8 a	71.9 a	40.3 a	37.6 a
	1.2–1.4	3.9 a	3.6 a	72.6 a	62.6 a	35.8 ab	12.5 b
LSD		2.28		30		24.43	
H3	0–0.2	4.7 ab	3.2 b	80.4 a	48.1 a	38.9 ab	21.6 b
	0.2–0.4	5.8 ab	5 ab	86.9 a	59.2 a	40.7 ab	26.4 b
	0.41–0.6	3.3 ab	4.1 ab	59.3 a	73.2 a	28.9 b	35.2 ab
	0.61–0.8	2.9 b	4.1 ab	45.8 a	74.3 a	20.3 b	33.7 ab
	0.81–1	5 ab	4.3 ab	68.6 a	68.7 a	28.1 b	25.5 b
	1.01–1.2	5.8 ab	9.9 a	81.4 a	69.5 a	37.2 ab	53.8 a
LSD		3.89		41.2		23.21	
Average	H1	4.0		71.9		27.6 b	
	H2	4.7		76.6		36.9 a	
	H3	4.9		67.9		32.7 ab	
	P value	0.3582		0.3017		0.0418	

Fruit were untreated (control) or treated with preharvest 1-methylcyclopropane (1-MCP) and harvested in three 1-week intervals. Different letters within the same parameter indicate significantly different means ($P < 0.05$), $n = 3$.

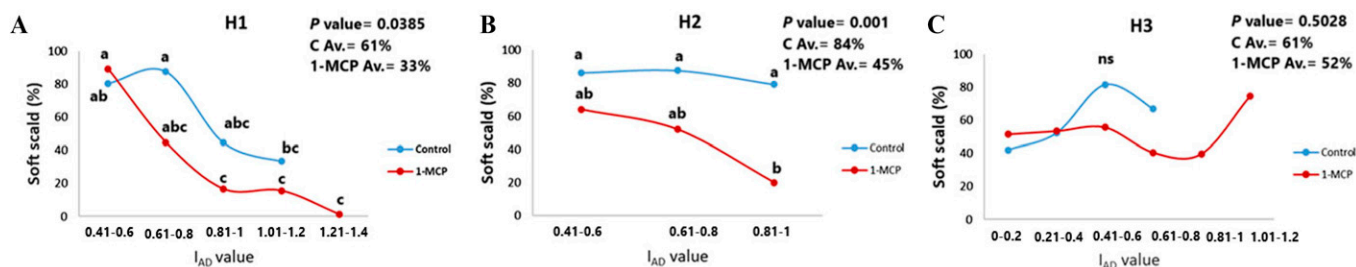


Fig. 3. Soft scald (%) in 'Honeycrisp' apples categorized by I_{AD} value at harvest. Fruit were untreated (control) or treated with preharvest 1-methylcyclopropane (1-MCP) and harvested at three 1-week intervals, H1 (A), H2 (B), and H3 (C). Different letters within the same figure indicate significantly different means ($P < 0.05$); $n = 517, 603$, and 568 for H1, H2, and H3, respectively.

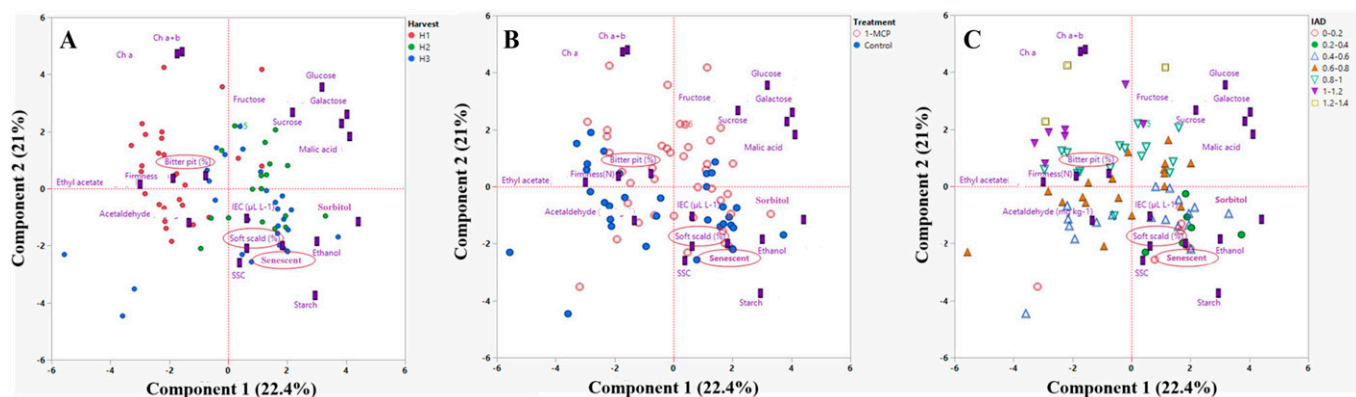


Fig. 4. Principal component analysis for harvest time (A), preharvest 1-methylcyclopropane (1-MCP) treatment (B), and I_{AD} category (C) for fruit maturity, sugars, and pigments at harvest and physiological disorder in 'Honeycrisp' apples after 4 months of storage at 0.5°C and 7 d at 20°C .

At H2 and H3, fermentation products increased at lower I_{AD} values, indicating advancing fruit maturity. This was evident with lower fermentation product accumulation in 1-MCP-treated fruit compared with untreated

fruit (Fig. 2). As ethanol can accumulate in apples if left on the tree and harvested late (Nichols and Patterson 1987), maintaining high I_{AD} values by preharvest 1-MCP suppresses ethanol production, especially in fruit from

late harvests. On the other hand, ester production in apple fruit is a process regulated by ethylene, with the majority of esters being synthesized during the climacteric phase of ripening (Song and Bangerth 1996). 1-MCP inhibited the ester ethyl acetate accumulation in fruit from all harvests, which aligns with various studies that found 1-MCP suppresses aroma volatiles in apple fruit and maintains higher fruit quality (Al Shoffe et al. 2024; Ferenczi et al. 2006).

The factors affecting sorbitol and sucrose translocation vary based on weather, nutrients, region, cultivar, and the fruit location in the canopy (Gao et al. 2005; Kviklyns et al. 2022). In our study, sucrose and sorbitol concentrations were increased by delaying the harvest time (Tables 3 and 5). However, the effects of sugar degradation to glucose and fructose in the cells and galactose in the cell wall in relation to ethylene content or fruit ripening on the trees require further research. Malic acid, the dominant acid in apple fruit (Hu et al. 2024; Zhang et al. 2010), is affected by postharvest 1-MCP treatment compared with untreated fruit. Fruit acidity also is higher in fruit treated with AVG, which extends the stability and longevity of the fruit, as malic acid is a key driver of fruit respiration (Lee et al. 2019).

In this study, we stored the fruit at 0.5°C to understand the occurrence of chilling injuries in relation to fruit maturity based on I_{AD} values given that soft scald is induced by storage at 0.5°C compared with 3°C (Watkins

Table 4. Pearson correlation for I_{AD} values against fruit maturity, quality, and physiological disorders of 'Honeycrisp' apples.

	I_{AD} range 0.4–1.4				I_{AD} range 0–1.2	
	H1		H2		H3	
	<i>r</i>	<i>P</i> value	<i>r</i>	<i>P</i> value	<i>r</i>	<i>P</i> value
IEC	−0.5211	0.0053	−0.3563	0.1467	−0.1664	0.3795
SPI	−0.7187	<0.0001	−0.7998	<0.0001	−0.8118	<0.0001
Firmness	−0.2476	0.213	−0.1532	0.5439	−0.0051	0.9786
SSC	−0.3532	0.0708	−0.4922	0.038	−0.6457	0.0001
Chl a + b	0.7945	<0.0001	0.6948	0.0014	0.8515	<0.0001
Chl a	0.8188	<0.0001	0.754	0.0003	0.8774	<0.0001
Chl b	0.3935	0.0423	0.0484	0.8486	0.3967	0.03
Fructose	0.4026	0.0373	−0.0574	0.821	0.1307	0.4912
Glucose	0.4537	0.0175	0.1617	0.5215	0.3008	0.1062
Sucrose	0.1178	0.5585	−0.1851	0.4621	0.1565	0.4088
Galactose	0.3358	0.0868	0.158	0.5313	0.2377	0.2059
Sorbitol	−0.299	0.1297	−0.1434	0.5704	−0.3097	0.0959
Malic acid	0.2483	0.2117	0.0127	0.9601	0.2147	0.2546
Soft scald	−0.7781	<0.0001	−0.5721	0.0131	0.0889	0.6403
Bitter pit	0.3585	0.0663	0.398	0.1019	0.2673	0.1533
Senescent	−0.3043	0.1228	0	1	−0.6607	<0.0001
Acetaldehyde	0.3122	0.1129	−0.2401	0.3371	−0.59	0.0006
Ethyl acetate	−0.3048	0.1221	−0.6647	0.0026	−0.5161	0.0035
Ethanol	0.3522	0.0716	−0.0995	0.6945	−0.1776	0.4954

Fruit were untreated or treated with preharvest 1-methylcyclopropane and harvested at three 1-week intervals. Fruit were stored at 0.5°C for 4 months and 7 d at 20°C . In every harvest time, the first column is for correlation (*r*), and the second column is for *P* value for I_{AD} categories against fruit maturity at harvest and fruit quality during storage. Correlation is significant when $P < 0.05$. Chl = Chlorophyll; IEC = internal ethylene concentration; SSC = soluble solids concentration.

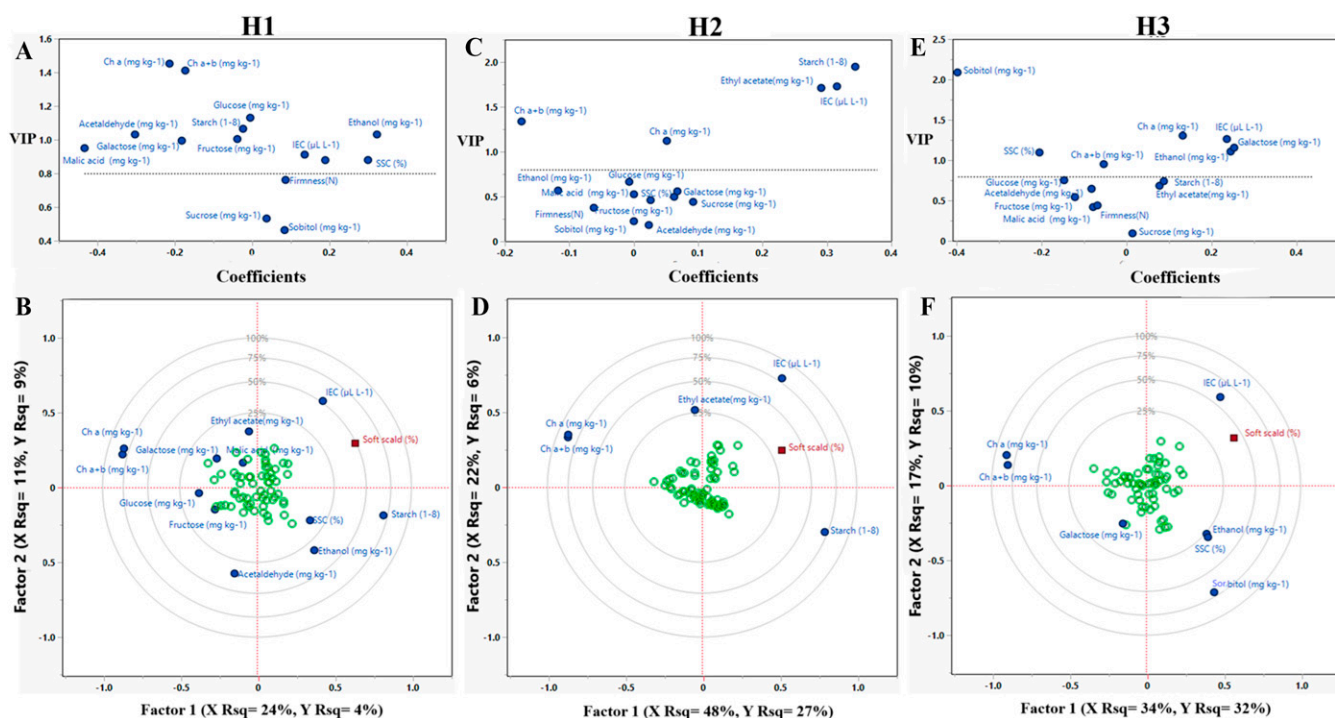


Fig. 5. Nonlinear iterative partial least squares (NIPALS) algorithm for regression of fruit maturity and quality at harvest against soft scald (%) for 'Honeycrisp' apples treated or not with preharvest 1-methylcyclopropene (1-MCP) and stored for 4 months at 0.5 °C and 7 d at 20 °C.

et al. 2004). Advanced fruit maturity based on the I_{AD} categories was associated with higher soft scald incidence of fruit at all harvests. In addition, 1-MCP treatment reduced soft scald development compared with untreated fruit in the first two harvests (Fig. 3). Previous studies showed that soft scald is associated with more mature fruit and that incidence was reduced by preharvest PGR treatment

(DeEll and Ehsani-Moghaddam 2010). Preharvest 1-MCP at different concentrations and timings mitigated soft scald incidence in 'Honeycrisp' apples compared with untreated fruit (Al Shoffe et al. 2021). While the effects of greater fruit maturity on soft scald incidence have been reported (DeEll and Ehsani-Moghaddam 2010; Moran et al. 2010; Sjöstrand et al. 2023), the relationship between

I_{AD} values and soft scald also has not been confirmed in these studies. In this study, we found that soft scald was associated with low I_{AD} values, being more pronounced in fruit at H1 and H2 (Table 4). Senescent breakdown incidence was low in our study since the storage at 0.5 °C reduces the fruit respiration, ethylene production, and metabolism. However, advancing fruit maturity increases fruit senescence during storage, resulting in a degradation of cell wall polymers, which leads to reduced integrity and, consequently, the softening of fruits. The most significant transformations are associated with the enzymatic degradation of pectins (Szymańska-Chargot et al. 2016).

To visualize the effects of the I_{AD} value in relation to fruit maturity and physiological disorders, we used PCA (Fig. 4). The figures illustrate the variability in fruit maturity and physiological disorder incidences associated with high I_{AD} values. I_{AD} values are strongly associated with IEC, SPI, chlorophyll, SSC, and soft scald incidence. This technology could be useful in sorting lines at harvest to predict fruit maturity and quality after storage for specific cultivars regulated by prediction models. Additionally, it is important to note that preharvest 1-MCP treatment can significantly reduce disorders during storage, but the timing of application is critical. We found that fruit from the third harvest did not benefit from the 1-MCP application, suggesting that delaying harvest beyond 3 weeks after application is not advantageous. More research is needed to optimize 1-MCP treatment to reduce physiological disorders in 'Honeycrisp' apples. Using modeling and regressions, we found that higher soft scald incidence was associated with higher IEC and SPI across H1

Table 5. Galactose, sorbitol, and malic acid concentrations of 'Honeycrisp' apples categorized by I_{AD} value at harvest.

Harvest	I_{AD} value	Galactose (g·kg ⁻¹)		Sorbitol (g·kg ⁻¹)		Malic acid (g·kg ⁻¹)	
		Control	1-MCP	Control	1-MCP	Control	1-MCP
H1	0.41–0.60	0.8 b	1.0 ab	1.8 ab	1.7 abc	5.6 a	5.3 a
	0.61–0.80	0.9 ab	1.1 ab	1.7 abc	2.1 a	5.7 a	6.6 a
	0.81–1	0.9 ab	1.2 ab	1.7 abc	1.7 abc	5.9 a	6.3 a
	1.01–1.2	1 ab	1.2 ab	1.5 abc	1.5 abc	6.1 a	6.1 a
	1.21–1.4	1.2 ab	1.5 a	1.1 c	1.4 b	5.4 a	6.7 a
	LSD	0.62		0.68		1.91	
H2	0.41–0.6	1.2 b	1.5 abc	3 abc	3.9 a	7.8 a	9 a
	0.61–0.8	1.1 c	1.5 abc	3 abc	3.1 abc	8.1 ab	8.2 ab
	0.81–1	1.4 abc	1.4 abc	2.5 bcd	3.4 ab	8.2 ab	8.4 a
	1.01–1.2	1.1 c	1.6 ab	3.2 ab	2 cd	8.7 a	9.0 a
	1.2–1.4	1.2 b	1.7 a	2.6 bcd	1.7 d	9.3 a	8.8 a
	LSD	0.46		1.18		0.58	
H3	0–0.2	1.4 ab	1.2 ab	3.7 ab	3.2 ab	7 a	5.4 a
	0.21–0.4	1.5 ab	1 b	3.7 ab	4.5 a	7 a	7.6 a
	0.41–0.6	0.9 b	1.4 ab	2.3 ab	3.4 ab	4.8 a	7.9 a
	0.61–0.8	1.0 b	1.4 ab	1.9 b	2.9 ab	5.1 a	7.7 a
	0.81–1	1.7 ab	1.4 abc	2.7 ab	2.6 ab	7.5 a	7.9 a
	1.01–1.2	1.6 ab	2 a	2.6 ab	2.5 ab	8.5 a	8 a
	LSD	0.94		2.45		3.86	
Average	H1	1.1		1.8 b		6.1 b	
	H2	1.3		3.1 a		8.3 a	
	H3	1.3		3.1 a		6.9 b	
	P value	0.0552		<0.0001		<0.0001	

Fruit were untreated (control) or treated with preharvest 1-methylcyclopropene (1-MCP) and harvested at three one week intervals. Different letters within the same parameter indicate significantly different means ($P < 0.05$), $n = 3$.

and H2 (Fig. 5). However, the role of galactose in increasing soft scald incidence is not well understood. Although our earlier work found that ethanol concentration is associated with soft scald development (Al Shoffe et al. 2023), in this study, ethanol was correlated with soft scald development only in fruit from the third harvest (Fig. 5).

Conclusions

The I_{AD} value is an effective indicator of fruit maturity in ‘Honeycrisp’ apples and can aid in harvest timing. Preharvest 1-MCP treatment mitigated chilling injury in ‘Honeycrisp’ apples. Across all harvest dates, IEC and the SPI had negative associations with I_{AD} values. Soft scald incidence was negatively correlated with I_{AD} values at H1 and H2, while ethyl acetate concentrations and the SSC were negatively correlated with I_{AD} at H2 and H3, the latter two harvests. Preharvest 1-MCP inhibited ethylene in H2 and ester production, maintained fruit acidity and chlorophyll concentration, delayed starch hydrolysis, and reduced soft scald development in early-harvested fruit while only slightly affecting sugar accumulation.

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