Patterns of starch hydrolysis in stem, equatorial, and calyx zones of ‘Honeycrisp’ and ‘Empire’ apples (Malus sylvestris (L.) Mill var. domestica (Borkh.) Mansf.) during maturation and ripening, and in ‘Gala’ apples in response to propylene or 1-methylcyclopropene (1-MCP) treatments after harvest, were studied. Differences in zonal starch concentrations were found for ‘Empire’ and ‘Gala’ fruits, but not for ‘Honeycrisp’. During maturation and ripening of ‘Empire’, the concentration of starch was highest in the calyx end and lowest in the stem region. Differences in rates of starch hydrolysis among zones were not detected. ‘Honeycrisp’ and ‘Empire’ had the highest concentration of sorbitol in the calyx region, whereas it was highest in the stem-end region in ‘Gala’. The distribution differences of glucose, fructose, and sucrose were similar in all three cultivars; higher fructose and glucose concentrations in the stem region, and higher sucrose concentrations in the calyx end of the fruit. Postharvest treatment of ‘Gala’ with propylene did not affect the internal ethylene concentration of the fruit but 1-MCP markedly inhibited it. Starch concentrations were highest in the calyx end but gradients of starch among zones were not changed by postharvest treatment. The rate of hydrolysis was slowed by 1-MCP treatment, but was unaffected by propylene. Postharvest treatments influenced sorbitol, glucose, and fructose concentrations. Patterns of starch concentration among the zones did not confirm differences in ripening, but reflected its uneven distribution throughout the fruit during development. Therefore, measured differences in zonal starch are most likely related to starch accumulation during fruit development, rather than differences in rates of starch degradation during ripening.

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degradation. Starch concentrations declined faster in ‘Tsugaru’ fruit treated with ethylene than in untreated fruit of the same cultivar.\textsuperscript{15,16} In contrast, rates of starch decline in ‘Fuji’ fruit, which generally produce low amounts of ethylene, were not affected by 1-MCP or ethylene treatments.\textsuperscript{15} ‘Tsugaru’ apples also showed few differences among treatments in total sugar concentrations among zones within the cortex tissue.\textsuperscript{16} Starch degradation appears to be very ethylene sensitive and starts before a substantial increase of internal ethylene concentration (IEC) is detectable.\textsuperscript{17}

Differences between starch concentrations and the rate of starch degradation within the fruit can be cultivar specific,\textsuperscript{18} but little information about differences in metabolism in stem- and calyx-end tissue of apples is available. In addition to presumed metabolic differences, physiological differences include higher tissue density in calyx-end than stem-end tissue,\textsuperscript{18} which collectively may have relevance to the development of physiological disorders such as flesh browning in ‘Empire’ and ‘Gala’ apples, which is first visible in the stem-end part of the fruit.\textsuperscript{20–23}

The objective of this study was to investigate changes in starch concentrations in stem-end, equatorial, and calyx-end zones of apple fruit. Two approaches have been taken. In the first, concentrations in different tissue zones of ‘Honeycrisp’ and ‘Empire’ fruit during maturation and ripening on the tree were measured. In the second, postharvest changes were measured in ‘Gala’ fruit kept at 20°C after treatment with propylene, an ethylene analogue,\textsuperscript{24} or 1-MCP.

**MATERIAL AND METHODS**

Maturation and ripening on the tree

‘Honeycrisp’ and ‘Empire’ apples (\textit{Malus sylvestris} (L.) Mill var. \textit{domestica} (Borkh.) Mansf.) were obtained from trees grown in the Cornell University Orchard, Ithaca, NY, USA. Five fruits of each cultivar were harvested weekly between August 16 and September 13, 2011, and thereafter twice a week until September 23 and October 18, respectively. Each fruit was analyzed separately. The IEC of each fruit was measured on each sampling date except the first (August 16), by injecting a 1-mL gas sample taken from the core cavity into a Hewlett-Packard 5890 series II gas chromatograph (Hewlett-Packard, Wilmington, DE, USA).\textsuperscript{25}

Tissue sampling

Tissue samples were then taken for analysis of soluble sugars and starch. Each fruit was cut latitudinally parallel to the core to leave an approximately 1-cm wide section around the core, stem, and calyx. Tissue samples were taken from the shoulder (stem), the equatorial cortex centered between core and skin, and the calyx-end of the fruit, using a core borer (diameter 1.9 cm). Tissues from the blushed and green sides of the fruit were combined to provide three samples per fruit: stem, equatorial, or calyx. The samples were frozen in liquid nitrogen and stored at −20°C until they were lyophilized. Dried samples were ground in a Wiley mill through a 20-mesh screen.\textsuperscript{26}

Postharvest manipulation of ripening

‘Gala’ apples were harvested from potted trees at the Cornell University Orchard in Ithaca, NY, USA, on August 29, 2012. In the lab they were split equally into three lots of ~60 fruit, and either untreated or treated with 1-MCP or propylene on the day of harvest. One set of fruit was put into a 4000-L plastic tent and treated with 1 μL L\textsuperscript{−1} 1-MCP (SmartFresh, AgroFresh Inc., Springhouse, PA, USA) for 24 h using a release and fan system supplied by the manufacturer. A second set was treated for 24 h with 200 μL L\textsuperscript{−1} propylene in a 280-L gas tight plexiglass chamber. Both treatments and the untreated control fruit were kept at 20°C. After removal from the chambers the fruit were allowed to vent before sampling 1, 2, 3, 5, 7, 9, and 13 days after harvest (DAH). Five randomly selected fruits were used at each sampling date, and analyzed separately. The IEC was measured as described above. \textit{IAD} was measured using a handheld non-destructive instrument (DA meter, Sinteleia, Bologna, Italy).\textsuperscript{27} Readings were taken on the opposite green and blushed sides and the average noted.\textsuperscript{28} Flesh samples were taken by cutting two wedges, each approximately one-eighth of the fruit. The wedge was peeled and placed on the edge of the cutting board where a 1-cm wide area was marked to ensure even cutting of the sections. The sections were frozen in liquid nitrogen and stored at −20°C until they were lyophilized and ground as described above.

Soluble carbohydrate and starch determination

For determination of sugars and starch, 50 mg of ground sample was washed with 3 mL 80% ethanol and incubated for 30 min at 70°C\textsuperscript{26,29} centrifuged (Eppendorf Model 5810 – Eppendorf North America, Inc., Westbury, NY, USA) and the aqueous phase separated into glass culture tubes. This was repeated three times, the extracts bulked and the aqueous phase containing all the soluble carbohydrates was run through columns of 1 mL Dowex 50-W, 100–200 mesh layered on 1 mL Amberlite IRA-45, 16–50 mesh\textsuperscript{26,29}. The column eluate was vacuum evaporated using a Rapidvap Vacuum Evaporation System (Labconco, Kansas City, MO, USA)\textsuperscript{30} for the maturation experiment (‘Honeycrisp’ and ‘Empire’), and an Evapo-Mix (Buchler Instruments, Fort Lee, NJ, USA) for the postharvest experiment (‘Gala’). The dry sugar samples were kept at −20°C until preparation for high-performance liquid chromatography. Maturation samples were dissolved in 10 mL high-performance liquid chromatography (HPLC) grade water and postharvest ‘Gala’ samples in 20 mL HPLC grade water.

In both experiments, the samples were further diluted by adding 400 μL HPLC grade water to 100 μL sample aliquots. Samples were analyzed using Dionex (Sunnyvale, CA, USA) high-performance anion exchange chromatography with pulsed amperometric detection (EDSO; Dionex) equipped with a Carbopac PA-1 column (CarboPac PA1 Analytical, 4 × 250 mm; Dionex). Carbohydrates were eluted with 100 mM NaOH at a flow rate of 1.0 mL min\textsuperscript{−1} for 20 min.\textsuperscript{30} Sorbitol, glucose, fructose, and sucrose were quantified by comparison with known standards.

The non-soluble carbohydrates obtained after extraction with ethanol were dried overnight at 60°C in the drying oven. Enzymatic starch determination with amyloglucosidase was performed.\textsuperscript{18,26,29}

**Statistics**

All statistical calculations were done in JMP Pro 11.0.0 (SAS Institute, Cary, NC, USA) using simple least-square models with inclusion of interactions. For regressions and analysis of variance (ANOVA) individual samples were used as replicates. ANOVA was performed on log-transformed IEC data, but back transformed and averaged IEC is presented within the graphs. Regressions within the graphs were calculated using OriginPro 8 SRO (OriginLab Corp., Northampton, MA, USA).

**RESULTS**

Maturation and ripening on the tree

Low levels of IECs were measured in ‘Honeycrisp’, except for a spike in ethylene on September 6 (Figure 1). ‘Empire’ IECs remained low throughout the experiment, and never exceeded 2.8 μL L\textsuperscript{−1} with the exception of one fruit on September 27 (120 μL L\textsuperscript{−1}). This fruit had likely absceded (‘push off’) and was not included in the analysis.

Starch concentrations per g dry matter in the stem-end, equatorial, and calyx-end zones of ‘Honeycrisp’ and ‘Empire’ decreased linearly during maturation and ripening (Figure 2). Differences in starch concentrations between zones were detected only for ‘Empire’ fruit. In this cultivar, concentrations were higher overall in the calyx-end (180 mg g\textsuperscript{−1}) than in the equatorial- and stem-end zones (171 mg g\textsuperscript{−1} and 141 mg g\textsuperscript{−1}, respectively). The daily decrease of starch was similar in all zones for each cultivar, ranging from 0.2 to 0.4% day\textsuperscript{−1}.
−6.33 mg g\textsuperscript{−1} to −6.96 mg g\textsuperscript{−1} for ‘Honeycrisp’, and −4.24 mg g\textsuperscript{−1} to −4.29 mg g\textsuperscript{−1} for ‘Empire’ (Figure 2).

Although there was variability among harvest dates, overall the concentrations of sorbitol, glucose, and sucrose increased in ‘Honeycrisp’ tissue zones over the harvest period, while those of fructose decreased (Table 1). Sorbitol and sucrose concentrations were highest in the calyx tissues, whereas glucose and fructose were highest in stem tissues. Similarly, the concentrations of sorbitol, glucose, and sucrose increased in ‘Empire’ tissue zones over the harvest period (Table 2). However, fructose concentrations changed over time, with a quadratic relationship being detected. Sorbitol and sucrose concentrations were highest in the calyx tissues, whereas glucose was highest in stem tissues. Fructose was not affected by tissue zone.

Table 1  Sorbitol, glucose, fructose, and sucrose (mg g\textsuperscript{−1} dry weight) in stem (S; ■), equatorial (E; ○), and calyx (C; ▲) at each harvest date.

| Harvest date (2011) | Sorbitol | Glucose | Fructose | Sucrose |
|---------------------|----------|---------|----------|---------|
|                     | S        | E       | C        | S        | E       | C        | S        | E       | C        |
| 8/16                | 8.2      | 10.4    | 10.3     | 45.3     | 29.9    | 17.3     | 303.7    | 293.1    | 281.9    | 202.7    | 196.8    | 228.3    |
| 8/24                | 8.9      | 10.1    | 13.7     | 57.8     | 51.6    | 30.7     | 274.7    | 286.0    | 280.0    | 215.1    | 225.8    | 252.2    |
| 8/30                | 11.9     | 14.7    | 15.2     | 52.0     | 40.8    | 34.4     | 282.1    | 270.5    | 272.9    | 241.9    | 259.3    | 272.6    |
| 9/06                | 12.7     | 15.2    | 18.7     | 60.4     | 42.4    | 30.5     | 318.7    | 285.5    | 274.1    | 288.9    | 292.3    | 291.0    |
| 9/13                | 11.7     | 15.0    | 18.7     | 61.8     | 39.9    | 31.3     | 282.5    | 273.1    | 288.2    | 279.0    | 319.6    | 357.7    |
| 9/20                | 14.6     | 21.0    | 22.6     | 48.4     | 40.6    | 33.5     | 266.9    | 247.4    | 253.9    | 324.4    | 333.2    | 350.5    |
| 9/23                | 10.2     | 12.0    | 13.4     | 62.6     | 51.0    | 43.3     | 249.2    | 236.6    | 239.2    | 379.8    | 397.1    | 407.3    |
| Overall means       | 11.2     | 14.1    | 16.4     | 55.0     | 40.0    | 31.7     | 284.6    | 271.8    | 271.8    | 269.7    | 284.2    | 305.9    |
| Regression          | L*       | L*      | L*       | ns       | L*      | L**      | L*       | L**      | L*       | L***     | L***     | L***     |
| Date                | <0.001   | 0.051   | <0.001   | <0.001   | <0.001  | <0.001   | <0.001   | <0.001   | 0.009    | 0.819    |          |          |
| Zone                | 0.002    | <0.001  | 0.071    |          | 0.819   |          |          |          |          |          |          |
| Date × zone         | 0.743    | 0.908   | 0.673    |          | 0.819   |          |          |          |          |          |          |

Each mean is the average of five fruits.
ns, *, **, *** = not significant or significant at P<0.05, 0.01, or 0.001, respectively
Postharvest manipulation of ripening

Propylene and 1-MCP were applied to ‘Gala’ apples with the objective of increasing and decreasing the rate of ripening, respectively. The IECs of fruit from all treatments remained low for the first 5 DAH (Figure 3). The IECs of untreated and propylene-treated fruit then increased, but no differences between them were detected. In contrast, the IECs of 1-MCP-treated fruit remained low throughout the experiment.

The decline in IAD values was slower in 1-MCP-treated fruit, indicating slower loss of chlorophyll a levels, compared with untreated fruit and those treated with propylene (Figure 4).

Regardless of treatment, starch concentrations were lowest in the stem-end tissues and highest in the calyx-end tissues, and these decreased linearly over time (Figure 5). 1-MCP-treated fruit generally had the highest concentration in each zone, while there was little difference between control and propylene-treated fruit. Although treatment with propylene led to lower starch concentrations per g dry matter combined for all zones and sampling dates.

### Table 2  Sorbitol, glucose, fructose, and sucrose concentrations (mg g⁻¹ dry weight) in stem (S), equatorial (E), and calyx (C) tissues of ‘Empire’ at each harvest date

| Harvest Date (2011) | Sorbitol (mg g⁻¹) | Glucose (mg g⁻¹) | Fructose (mg g⁻¹) | Sucrose (mg g⁻¹) |
|---------------------|-------------------|-----------------|------------------|-----------------|
|                     | S                 | E               | C                | S               | E   | C     | S     | E   | C   | S     | E   | C     |
| 8/16                | 10.5              | 12.3            | 14.4             | 44.2            | 23.2 | 22.3             | 266.1           | 277.7          | 263.1           | 137.1 | 154.5       | 164.0 |
| 8/24                | 10.6              | 14.6            | 17.7             | 43.6            | 32.5 | 31.4             | 301.0           | 299.7           | 286.8           | 149.2 | 165.0       | 169.0 |
| 8/30                | 12.7              | 14.8            | 17.7             | 59.8            | 40.2 | 38.5             | 280.7           | 281.6           | 272.3           | 164.9 | 192.3       | 200.8 |
| 9/06                | 9.5               | 12.6            | 13.9             | 53.5            | 38.6 | 37.4             | 253.4           | 287.1           | 278.9           | 154.6 | 190.6       | 196.9 |
| 9/13                | 15.5              | 16.7            | 17.7             | 75.6            | 56.7 | 46.6             | 256.1           | 282.4           | 260.2           | 209.5 | 236.7       | 240.3 |
| 9/20                | 18.6              | 24.6            | 25.6             | 75.6            | 56.7 | 46.6             | 256.1           | 282.4           | 260.2           | 209.5 | 236.7       | 240.3 |
| 9/23                | 11.2              | 16.4            | 16.1             | 54.9            | 35.5 | 30.5             | 275.6           | 281.7           | 274.3           | 220.6 | 239.4       | 251.6 |
| 9/27                | 13.6              | 17.4            | 20.7             | 63.1            | 46.1 | 43.1             | 279.2           | 267.9           | 279.6           | 232.2 | 236.3       | 261.3 |
| 9/30                | 12.3              | 14.7            | 15.0             | 55.0            | 42.3 | 36.9             | 277.9           | 256.0           | 255.3           | 237.0 | 254.6       | 258.1 |
| 10/04               | 13.6              | 19.4            | 20.2             | 78.6            | 55.7 | 48.6             | 322.6           | 320.3           | 310.4           | 216.8 | 255.8       | 278.8 |
| 10/07               | 16.5              | 25.4            | 24.6             | 69.6            | 57.2 | 49.8             | 309.5           | 302.0           | 285.2           | 254.4 | 287.0       | 288.8 |
| 10/14               | 15.5              | 24.9            | 23.6             | 64.8            | 51.1 | 46.6             | 257.0           | 274.6           | 251.4           | 252.8 | 293.2       | 289.8 |
| 10/18               | 17.1              | 18.2            | 18.2             | 56.4            | 52.7 | 46.1             | 248.7           | 245.0           | 229.7           | 287.0 | 284.0       | 282.8 |
| Overall means       | 13.7              | 17.8            | 18.8             | 60.2            | 43.9 | 39.5             | 279.5           | 281.5           | 271.4           | 208.3 | 227.6       | 236.2 |

Each mean is the average of five fruits.
ns, *, **, *** = not significant or significant at P < 0.05, 0.01, or 0.001, respectively

P-values

Date ± 0.001 Zone ± 0.001 Date ± 0.001

Zone ± 0.01 Date ± 0.001

Each mean is the average of five fruits.
with 53 mg g\textsuperscript{-1} compared with 57 mg g\textsuperscript{-1} and 69 mg g\textsuperscript{-1} for control and 1-MCP-treated fruit, respectively; a statistical difference between the propylene-treated fruit and the untreated was not detected. Starch concentrations in the calyx-end of the fruit over all treatments were 34% higher compared with the equatorial zone, and 74% higher compared with the stem-end tissues.

Concentrations of sorbitol generally decreased, while those of sucrose generally increased over time, although the patterns were curvilinear (Figure 6; Table 3). Sorbitol in untreated fruit decreased between 1 DAH and 13 DAH in the stem, equatorial and calyx zones decreased by 36%, 67% and 50%, respectively. Propylene-treated fruit had lower sorbitol concentrations: 43%, 37% and 50% for stem-, equatorial-, and calyx-end zones, respectively. Sorbitol in 1-MCP-treated fruit decreased until 9 DAH, but increased at the last sampling point (13 DAH). In contrast to the general trend of a continuous concentration gradient, the equatorial zone sorbitol was slightly higher than stem-end concentrations on most sampling days for all treatments. Glucose increased in untreated control and 1-MCP-treated fruit, but remained relatively level in propylene-treated fruit. The glucose concentrations of control and 1-MCP-treated fruit on day 13 were higher (average of zones being 60 mg g\textsuperscript{-1} and 64 mg g\textsuperscript{-1}, respectively) compared with that in propylene-treated fruit (52 mg g\textsuperscript{-1}). Fructose concentrations increased linearly over time. Zonal effects on fructose concentrations were most pronounced in 1-MCP-treated fruit at day 13 with equatorial and calyx at 160 mg g\textsuperscript{-1} and 157 mg g\textsuperscript{-1} compared with the stem-end with 176 mg g\textsuperscript{-1}. Little differences in fructose concentrations were found between control- and propylene-treated fruit on day 13, but overall concentrations were higher in the stem-end zone compared with the equatorial zone, and lowest concentrations were in calyx, averaging 135 mg g\textsuperscript{-1}, 135 mg g\textsuperscript{-1}, and 130 mg g\textsuperscript{-1} in stem, equatorial, and calyx zone of control,

Figure 5  Starch concentration (mg g\textsuperscript{-1} dry wt) in stem, equatorial, and calyx tissues of ‘Gala’ in untreated control (C; □), propylene-treated (P; ●), or 1-MCP-treated (M; ▲) fruit from at harvest (0) to 13 DAH (left side) and (right side) starch concentration in fruit zones of either untreated control, propylene, or 1-MCP-treated fruit; stem-end (S; ■), equatorial (E; ○), and calyx-end (C; ▲). Each mean is the average of five fruits. The $R^2$ describes the linear fit of the data; $P$-values: treatment < 0.001, zone < 0.001, DAH < 0.001, zone $\times$ DAH < 0.001, no other interactions detected.
Sucrose concentrations varied among zones with the highest concentration in the calyx, and they reached maximum values at different times depending on treatment. Zones of propylene-treated fruit all reached the maximum at 9 DAH. Equatorial and calyx-end zones of control and 1-MCP-treated fruit also reached highest sucrose concentrations on 9 DAH, but the calyx-end concentration increased until 13 DAH. The effect of tissue treatment on sucrose content is illustrated in Figure 6.
Table 3  ANOVA for sorbitol, glucose, fructose, and sucrose tissue zones (stem, equatorial, and calyx) of ‘Gala’ with treatment (untreated (control), propylene or 1-MCP), zone, and days after harvest (DAH)

| Treatment x zone | Sorbitol | Glucose | Fructose | Sucrose |
|------------------|----------|---------|----------|---------|
| DAH              | 0.001    | 0.031   | 0.049    | 0.288   |
| Treatment        | <0.001   | <0.001  | <0.001   | <0.001  |
| Zone             | 0.944    | 0.989   | 0.952    | 0.938   |
| Treatment x DAH  | <0.001   | 0.131   | <0.001   | <0.001  |
| Zone x DAH       | 0.879    | 0.072   | 0.597    | 0.001   |
| Treatment x zone x DAH | 0.986 | 0.653  | 0.874  | 0.964 |

zone was significant for all sugars and an interaction between tissue zone and DAH was detected for sucrose (Table 3). The interaction between treatment and DAH was significant for sorbitol, fructose, and sucrose.

**DISCUSSION**

Starch concentrations in all tissue zones declined in a linear fashion over time, regardless of cultivar, and whether fruit were on or off the tree. These patterns of change are therefore consistent with those found for starch concentrations in whole fruit. Rates of decrease were similar between the same zones in the different treatments, but were different within fruit of the same treatment. Nevertheless, this might not indicate differences in rates of ripening per se, but rather may reflect differences in starch accumulation in different tissue zones during fruit development. Although starch is known to accumulate during fruit development to a maximum before net starch loss occurs, little is known about rates of deposition in different fruit tissue. Starch concentrations in the calyx-end tissue in all treatments of ‘Gala’ were higher at 1 DAH compared with those in the equatorial and stem zones (Figure 5). ‘Empire’ fruit also had a higher starch concentration in the calyx-end than in the stem-end (Figure 2). Higher amounts of starch in the outer cortex compared to the core and the vascular bundle region were previously found by Brookfield et al.

The effect of higher IEC on starch has been shown previously, with a hastening effect of increased ethylene on starch hydrolysis. However, Thammawong and Arakawa found that exogenous ethylene increased the rate of starch hydrolysis only in ‘Sugarsu’ not in ‘Fuji’ fruit. Relative independence of SPI changes in fruits treated with ethylene was also observed by Blankenship and Unrath in ‘Golden Delicious’. Starch concentrations in ‘Gala’ treated with propylene were slightly lower in all three tissue zones compared with the untreated control fruit. Propylene treatment did not elevate IEC, and a higher dose of, or prolonged exposure to, propylene might have been needed to increase IEC sufficiently and affect starch hydrolysis. Little effect of 1-MCP on starch degradation was detected in ‘Jonagold’ and ‘Golden Delicious’ apples supporting the idea that starch hydrolysis is relatively ethylene independent. Initiation of starch hydrolysis is very ethylene sensitive; however, and therefore even a very small increase in IEC can trigger the onset of starch hydrolysis. Once initiated the progression is relatively independent of ethylene concentrations within the fruit tissue. In ‘Gala’, starch degradation occurred even with low IECs in 1-MCP-treated fruit; hydrolysis may have been already initiated on the tree. The effects of 1-MCP on decreasing starch hydrolysis in ‘Gala’ were stronger than those observed for ‘Jonagold’ and ‘Golden Delicious’, but those observations were based on SPI readings and not chemical analyses of starch.

Starch hydrolysis has been shown to initiate and proceed simultaneously throughout the fruit and higher initial values of starch in the calyx-end of the fruit (Figures 2 and 5) supports the assumption that starch is not evenly distributed within the fruit during development. Such distributional differences within the fruit earlier in development with changes in granule size or granule structure have been documented. Granules are degraded from the inside out, leading to a rate of degradation not related to granule size. The actual structure and composition of starch was not analyzed here, but changes in starch composition of all three cultivars were previously studied in the whole fruit. The percentage amylose (linear starch) in total starch decreases as the fruit matures. This also supports the assumption that differences within the starch granule cause differences in starch degradation. Therefore, the differences in granule size and/or number of starch granules in different fruit tissue zones could lead to differences in starch distribution within the fruit, even if starch degradation is initiated simultaneously in the entire fruit. Hence, the measured differences could be artifacts from earlier events during fruit development rather than differentiated starch degradation.

Low glucose and sorbitol, and increasingly higher concentrations of sucrose and fructose during fruit ripening and storage have been described. Changes in sugar concentrations in the whole fruit (on a fresh weight basis) have been seen in ‘Honeycrisp’ during development. Similar trends of sugar accumulation were measured in the current study; all three cultivars had low concentrations of sorbitol and glucose, and much higher concentrations of fructose and sucrose, which increased as the fruit ripened. Sorbitol is the preferred transport sugar in Rosaceae fruit trees, but generally represents a relatively small proportion of the total carbohydrates in the fruit. Sorbitol is not stored, but is converted into other sugars, mostly fructose by NAD-dependent sorbitol dehydrogenase. Therefore, the already low levels of sorbitol decrease after harvest, since there is no longer an influx of sorbitol. Fluctuations in glucose levels could be explained since glucose is used for respiration and is transformed into fructose and subsequently sucrose (sucrose-6-phosphate-synthase). This could explain the small increase in glucose compared with the hydrolysis of starch and the much greater increase in fructose and sucrose. Glucose is not as sweet in taste as fructose and sucrose and, therefore, does not contribute as much to flavor characteristics of the fruit. The sucrose concentration gradient between tissue zones was similar to the starch concentration gradient, with higher concentrations in the calyx region. The other sugars show an opposite trend in ‘Gala’, with higher levels in the stem region. Differences in sugar concentration between parts of the apple fruit have been documented previously with higher total sugar concentrations in the stem-end vs. the calyx-end of the fruit, similar to those sugar concentrations found in ‘Honeycrisp’, ‘Empire’, and ‘Gala’. However, variations between fruits are much greater than variance of total sugars within the fruit.

‘Gala’ fruit treated with 1-MCP maintained higher IAD values, indicating slower chlorophyll a degradation by the treatment. Hue angle of skin has been found to have low sensitivity, and therefore stronger dependence on ethylene. Propylene-treated fruit decreased in IAD at a similar rate as untreated fruit, but the values of IEC were also very similar. Delay of chlorophyll a degradation by 1-MCP during storage has been found previously.

A zonal gradient of starch and sugar concentration between the stem- and calyx-end of the fruit was shown in ‘Empire’ and ‘Gala’ fruit. The results for ‘Honeycrisp’ were not as conclusive. Whether these differences are purely due to a distributional difference in starch accumulation during development and therefore an artifact of starch distribution during fruit growth or whether this proves a differentiation in maturation among tissue zones cannot be answered based on these data alone. More research needs to be carried out to understand the developmental differences between the stem and calyx region of the fruit. Changes in starch and soluble sugars cannot verify true maturity differences, and may be artifacts from the earlier development of larger starch granules in the lower
part of the fruit. Also, IECs of 'Gala' fruit treated with propylene or 1-MCP alone could not explain the differences in starch decline between the zones and/or treatments, so there still could be a linkage between IEC and starch hydrolysis. Very small amounts of ethylene trigger the onset of starch hydrolysis,
17 increasing the amount of ethylene hastens the progression. Such effects of ethylene could not be detected in our study but the propylene treatment, without affecting overall IECs, did lead to a lower starch amount of ethylene hastens the progression. Such effects of ethylene.

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