

Nondestructive Apple Ripening Stage Determination Using the Delta Absorbance Meter at Harvest and after Storage

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ADDITIONAL INDEX WORDS. *Malus domestica*, ripening index, fruit quality, VIS spectroscopy, DA meter, ethylene, predictive model

SUMMARY. The delta absorbance (DA) meter is a handheld instrument which noninvasively measures the chlorophyll content in fruits. In the present work, it was used to monitor the ripening process linked to the climacteric phase in apple (*Malus × domestica*). The results [index of absorbance difference (I_{AD})] were correlated to quality attributes at harvest and after commercial scale storage at different conditions. Two cultivars (Red Delicious, Golden Delicious) were analyzed in two different seasons, whereas Morgenduft and Gala were analyzed only in the first and second seasons, respectively. In general, a linear reduction of the I_{AD} values was observed in all apple cultivars along with the progression of ripening and ethylene biosynthesis. When ethylene production was inhibited by 1-methylcyclopropene (1-MCP) treatment, the decrease of I_{AD} values was markedly reduced. I_{AD} threshold values for each cultivar were identified, delineating the central phase of the ethylene climacteric rise. Predictive models were built by correlating I_{AD} index to the soluble solids concentration (SSC), titratable acidity (TA), and firmness measured at harvest and after removal from different storage regimes. The best model was developed for SSC prediction on ‘Red Delicious’ apple [ratio performance deviation (RPD) = 1.88] and for firmness evaluation in ‘Golden Delicious’ apple (RPD = 1.84). Moreover, I_{AD} values were consistently associated with the differences in fruit quality as affected by optimal and suboptimal storage conditions. The I_{AD} , due to its acceptable accuracy and speed of assessment, can be a promising tool for assisting in sorting apples before and after storage in warehouses or commercial packing lines. I_{AD} cannot totally replace standard ripening indices, but can effectively supplement data for these parameters.

An effective marketing program for apples requires the production and distribution of fruit at high and uniform quality levels to both satisfy consumer demands and generate repeated purchases. Although each market segment may define quality differently, the components of apple quality are usually measured in terms of fruit appearance, taste, and internal conditions. Harvest date is an essential factor affecting apple quality after storage. Even the best postharvest techniques can only maintain fruit quality, not improve it. Therefore, an accurate assessment of harvest maturity is essential. Commonly used harvest

indices are based on skin background color, fruit flesh firmness, starch pattern index, SSC, TA, and ethylene production (Kingston, 1992), but no one parameter gives a completely reliable measure of harvest readiness. Consequently, the ripeness evaluation usually needs to combine two or more indices. Ripening parameters are generally determined through destructive methods, with several disadvantages. These techniques are often inefficient, time consuming, require preparation of sample, and use of chemical products. Moreover, the

analysis is performed on a few samples that are often not completely representative of the variability within the fruit lots. Destructive analysis techniques do not enable monitoring the physiological changes on the same samples over the entire ripening period. Hence, nondestructive sensing techniques capable of measuring maturity/quality parameters may be very useful in determining optimal harvest time and ensuring high-quality products. In this regard, the use of spectroscopic methods for the evaluation of product quality is very promising because it is simple, fast, nondestructive, and therefore applicable to a great number of samples.

A precise way to determine the onset of the ripening process without fruit destruction is by monitoring the respiration rate or the ethylene production (Wills et al., 1998). The ripening of apple, as all climacteric fruit, is determined by the production of ethylene, an important phytohormone responsible for changes in the overall quality of fruit (Ireland et al., 2014). The drawbacks of these techniques are the expense of instruments, the time needed to perform the analysis and the need for skilled personnel.

During apple development, there is a trend in chlorophyll reduction along with progression of fruit maturity. The decrease in chlorophyll concentration may therefore be considered as an index of the fruit development stage as well as of fruit quality (Merzlyak et al., 1999) and has been used by McGlone et al. (2002) to determine the optimum harvest period. The chlorophyll content of the fruit skin provides the ground color of fruit (Blanck and Notton, 1992; Jacob-Wilk et al., 1999). Nevertheless, visual assessments like background color evaluation and the use of color cards do not provide an effective identification of

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| Units | | | |
|------------------------------------|-----------|---------------------|------------------------------------|
| To convert U.S. to SI, multiply by | U.S. unit | SI unit | To convert SI to U.S., multiply by |
| 29.5735 | fl oz | mL | 0.0338 |
| 3.7854 | gal | L | 0.2642 |
| 25.4 | inch(es) | mm | 0.0394 |
| 4.4482 | lbf | N | 0.2248 |
| 1 | ppb | µg·kg ⁻¹ | 1 |
| 0.001 | ppm | g·L ⁻¹ | 1000 |
| 1 | ppm | µL·L ⁻¹ | 1 |
| (°F - 32) ÷ 1.8 | °F | °C | (°C × 1.8) + 32 |

the fruit ripening stage, relying on subjective comparison against rather imprecise discrete scales. Instrumental monitoring of fruit skin color represents a potential tool for estimating fruit ripening stage. Tristimulus color measurements have been proposed as an alternative for background color evaluation, although in some cases such measurements have been reported to correlate poorly with pigment composition (Lancaster et al., 1997).

Although chlorophyll concentration of the skin concurs with the apple ground color, it is often masked early on by the anthocyanins in red apple cultivars. Thus, visual assessment of skin color might not precisely identify the fruit ripening stage. Visible/near IR (Vis/NIR) spectroscopy allows the evaluation of the chlorophyll concentration even in the skin of red apples whose anthocyanin content is already high from the first stages of maturation, thus providing the dominant blush color (Bertone et al., 2012).

The feasibility of using the chlorophyll concentration as a fruit ripening marker has been demonstrated by correlating pigment content to the respiration rate and ethylene production in apple and peach [*Prunus persica* (Ziosi et al., 2008; Zude-Sasse et al., 2000)]. For both fruits, evaluation of chlorophyll concentration has been used for the analysis of the fruit ripening stage (Bertone et al., 2012; Eccher Zerbini et al., 2006; Solovchenko et al., 2005; Tijskens et al., 2007; Ziosi et al., 2008; Zude-Sasse et al., 2002).

The handheld DA meter (Sinteleia, Bologna, Italy) measures chlorophyll concentration nondestructively and gives an I_{AD} . It was developed from a study on peach and nectarine (*P. persica*) maturation (Ziosi et al., 2008) and then was used on plum [*Prunus domestica* (Infante et al., 2011b)], apricot [*Prunus armeniaca* (Costa et al., 2010)], and peach post-harvest management (Spadoni et al., 2016). The I_{AD} characterizes the changes in fruit ripening on the basis of a calculation of absorption difference between 670 and 720 nm. This is close to the absorption peak of chlorophyll *a* normalized by subtracting an absorbance that does not change with chlorophyll breakdown. The same system also has been used

to identify the optimal harvest period for different apple cultivars (DeLong et al., 2014, 2016; Doerflinger et al., 2016) and for the determination of internal quality attributes of apples at harvest and after storage in different atmospheres, with or without 1-MCP treatment (Costamagna et al., 2013; Nyasordzi et al., 2013; Toivonen and Hampson, 2014). Information is lacking about I_{AD} evaluation through the apple ripening process, directly monitored by ethylene production throughout the period of the climacteric phase, and of apple quality characteristics determined on fruit picked from several orchards spread over a large area, at harvest and after storage on a large commercial scale, using controlled atmosphere (CA), and 1-MCP technologies.

In the present study, the feasibility of I_{AD} prediction of apple maturity stage has been evaluated by coupling DA meter measurements to ethylene production during the progression of ripening after harvest and to quality/maturity indices, before and after different cold storage. Predictive models were built by correlating I_{AD} to SSC, TA, and firmness measured at harvest and after storage. The work was carried out with the apple cultivars Red Delicious, Golden Delicious, Gala, and Morgenduft over 2 years.

Materials and methods

Fruit material and experimental setup

Apples of the cultivars Red Delicious, Golden Delicious, Gala, and Morgenduft were picked during the commercial harvest period from different orchards in the surroundings of Sondrio, in the Valtellina area, Italy (lat. 46°10'00"N, long. 9°52'00"E). The harvest dates of the 2 years of the experiment (2012–13) were 12 Oct. and 28 Sept. for 'Red Delicious', 12 Oct. and 11 Oct. for 'Golden Delicious'. 'Morgenduft' was sampled only the first year on 12 Oct. 'Gala' only the second year on 28 Aug. A packinghouse in Valtellina that collects produce from farms spread across the area, provided fruit on the day of harvest for the two different trials both performed in 2012 and 2013.

TRIAL 1. I_{AD} changes and ethylene production during the climacteric. The first trial dealt with

ethylene production and I_{AD} determination during 27 d of shelf life at 20 °C. Immediately after the samples were collected, the fruit of 'Morgenduft' (2012), 'Red Delicious', 'Golden Delicious' (2012 and 2013), and 'Gala' (2013) were brought to the laboratory in Milan, numbered, weighed, and the I_{AD} index was measured on each fruit. Then the apples were divided into two groups: 1) 15 fruit for destructive analysis of quality parameters and 2) 15 apples were maintained at 20 °C and in the second year, another batch of 15 apples of each cultivar was added to the trial after treatment at the packinghouse with 1-MCP (Smart Fresh®; Agro-Fresh, Philadelphia, PA) on the day of harvest. The treatment was applied in a storage room at the concentration of 1 $\mu\text{L}\cdot\text{L}^{-1}$ for 12 h at 20 °C, using tablets and associated release solutions. The two groups of 15 untreated and 1-MCP-treated fruit were maintained at 20 °C for 27 d.

TRIAL 2. Effect of storage conditions on I_{AD} changes and quality attributes. In the second trial, I_{AD} and quality parameters were measured immediately after harvest as well as after a cold storage period. Different storage conditions were tested to describe the normal post-harvest handling of apples in commercial packinghouses under optimal (CA) and suboptimal (air) storage atmospheres. 'Red Delicious' and 'Golden Delicious' were used in both years for the trial; 'Gala' was used only in the year 2.

Seventy-five fruit of 'Red Delicious' or 'Gala' were divided into three groups. Twenty-five apples of each cultivar were immediately used to carry out destructive analyses of quality attributes (Group 1). After weighing and I_{AD} measurement, 25 untreated apples were placed in storage at 1 °C, 95% relative humidity (RH) under air conditions, in storage rooms of the packinghouse (Group 2). Twenty-five apples of each cultivar were stored under CA [1% oxygen (O_2), 2.5% to 3% carbon dioxide (CO_2), 94% to 99% RH, 1 to 1.5 °C] by applying Smart Fresh® technology (1-MCP) in the same facility (Group 3). For 'Golden Delicious', 45 apples were split into the same above-mentioned groups (15 apples each). The duration of cold storage

(in both storage regimes) was 4 months for ‘Gala’ and 6 months for ‘Red Delicious’ and ‘Golden Delicious’ in both years. The stored apples underwent the same parameter evaluations performed at harvest.

Nondestructive analysis of fruit: I_{AD} and ethylene production

I_{AD} was measured daily on apples during shelf life at 20 °C (trial 1), whereas in trial 2, I_{AD} was measured at harvest and immediately after cold storage. I_{AD} measurements were taken on two opposite positions (sun-exposed and shaded sides) on the equator (marked with a circle) of each apple. In this way, all measurements were carried out exactly at the same position on the fruit. Fruit tested after cool storage were maintained at 20 °C for 6 h to allow for acclimatization to the experimental conditions.

Ethylene production was monitored daily during the ripening process at 20 °C by withdrawing a 1-mL headspace gas sample with a syringe from 1-L airtight jars. Each jar held one fruit that was enclosed for 1 h at 20 °C. The gas was injected into a gas chromatograph (model 3800; Dani Instruments, Cologno Monzese-Milano, Italy) and analyzed as previously described (Benedetti et al., 2008). I_{AD} values were recorded daily throughout the shelf life period and were plotted together with the corresponding rates of ethylene production, also measured daily. I_{AD} threshold values that delineate the central phase of the climacteric were determined in correspondence to the maximal increase/decrease in the rate of ethylene production.

To correlate the I_{AD} to the ethylene production, I_{AD} was measured throughout the ripening period on the same apples and on the same skin area. This strategy provided more precise information about the fruit ripening behavior over time and ensured synchronized data for changing I_{AD} and ethylene evolution. Moreover, during this trial only the fruit side with the higher I_{AD} values (i.e., sun-exposed in red cultivar and shaded in Golden Delicious) was taken into account, to have good index measurements in ‘Gala’, whose I_{AD} values were already low at the beginning of the shelf life period and because the trends of I_{AD} decrease

were similar for both fruit sides. In all the other cases, i.e., to set up the linear regression (LR) models and to evaluate quality parameters after different storage conditions, I_{AD} measurements were taken at two equatorial opposite points on each fruit and the average of the two measurements was calculated.

Destructive analysis of fruit: ripening/quality parameters

At harvest and after storage, the following destructive measurements were performed on each fruit: firmness, SSC, and TA.

Firmness was measured on two opposite peeled sides of each fruit by a pressure tester (EFFE.GI, Ravenna, Italy) fitted with an 11-mm plunger.

SSC was determined with a temperature-compensating digital refractometer (Atago, Tokyo, Japan) for juice samples extracted by squeezing cortical wedges cut from two opposite sides of each fruit. Two measurements were obtained for each fruit and averaged to give percentage SSC.

TA was determined on 10 mL of juice extracted from two different fruit. The juice was titrated to an endpoint at pH 8.1 with 0.1 N sodium hydroxide (NaOH) (Compact-S Titrator; Crison, Modena, Italy) and values are reported as malic acid in grams per liter.

Statistical analysis

The relationships between I_{AD} and ripening/quality data were analyzed with SPSS software Statistics 22 (IBM Segrate, Milano, Italy) for analysis of variance, and means were compared with Tukey test at $P=0.05$. The correlations between I_{AD} and other quality parameters were performed, using data of samples before (i.e., immediately after harvest) and after storage in CA, that is in optimal, commercial conditions in both years for ‘Red Delicious’ and ‘Golden Delicious’. Calibration models were developed for the ripening/quality parameters closely linked to the ripening/quality perception by the consumer (firmness, SSC, and TA) using The Unscrambler® 9.8 software package, Linear Regression algorithm (CAMO, Oslo, Norway). Conversely, a predictive model for the ethylene prediction was not calculated as the same ethylene values may correspond to different ripening stages, because

of its pattern characterized by a curve with a central peak. Due to the limited number of samples in the data set, cross-validation was applied as the validation method (leave-more-out, cancellation groups with five elements). To evaluate model accuracy, the coefficient of determination in calibration, the root mean standard error of calibration, the coefficient of determination in cross-validation, and the root mean standard error of cross-validation (RMSECV) were calculated. Finally, for each elaborated model, the RPD was calculated. RPD is defined as the ratio between the standard deviation of the response variable and RMSE in validation. An RPD ratio less than 1.5 indicates incorrect predictions and the model cannot be used for further prediction. An RPD between 1.5 and 2 means that the model can discriminate low from high values of the response variable; a value between 2 and 2.5 indicates that coarse quantitative predictions are possible, and a value between 2.5 and 3 or above corresponds to good and excellent prediction accuracy, respectively (Williams and Sobering, 1996).

Results

I_{AD} AND ETHYLENE PRODUCTION. I_{AD} values decreased progressively throughout 27 d at 20 °C. I_{AD} values for ‘Red Delicious’ and ‘Gala’ registered on the fruit side exposed to solar radiation were always higher than those of the shaded side (Fig. 1). In contrast, I_{AD} measurements of the yellow ‘Golden Delicious’ were higher on the shaded fruit side. Nevertheless, differences between daily averages of I_{AD} measured on the two sides of the apples were not statistically significant, except for ‘Gala’. At harvest, ‘Red Delicious’ displayed the highest I_{AD} values (ranging from 1.78 to 1.34), followed by ‘Morgenduft’ (1.77 to 1.59), ‘Golden Delicious’ (1.34 to 0.87), and then ‘Gala’ (0.84 to 0.39). During shelf life, the decrease in I_{AD} values was steeper in ‘Morgenduft’ (data not shown) and ‘Red Delicious’, with an average decrease in 27 d of 1.19 and 1.17, respectively. Moreover, the differences between I_{AD} values measured at harvest and at the end of shelf life were about 0.87 in ‘Golden Delicious’ and 0.30 in ‘Gala’. The gradual decline in I_{AD}

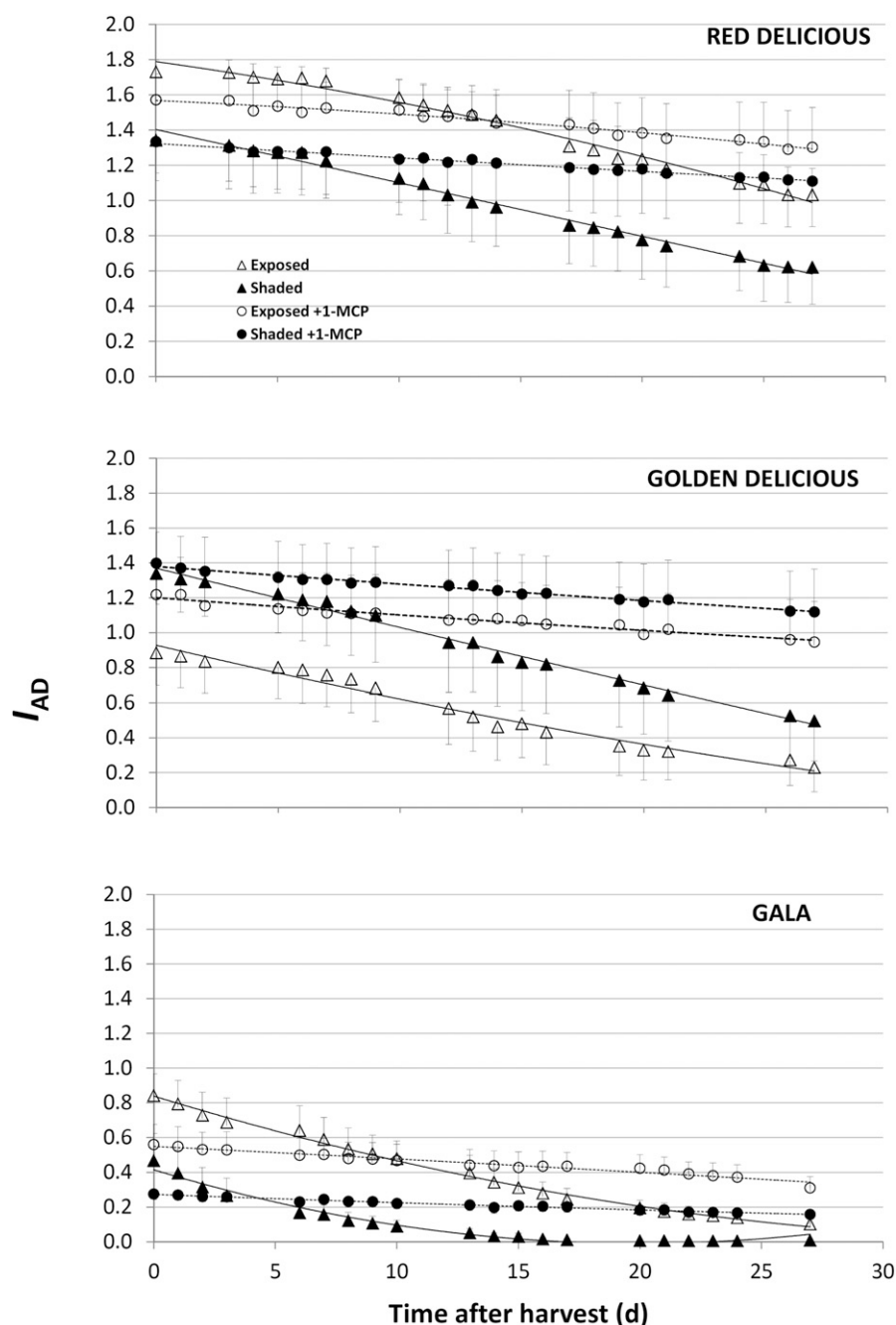


Fig. 1. Index of absorbance difference (I_{AD}) during apple shelf life at 20 °C (68.0 °F). Values are recorded in the second season on sun-exposed and shaded sides of ‘Red Delicious’, ‘Golden Delicious’, and ‘Gala’ apples not treated or treated with 1-methylcyclopropane (1-MCP) at the concentration of 1 $\mu\text{L}\cdot\text{L}^{-1}$ (ppm) for 12 h at 20 °C. Vertical bars represent $\pm\text{SE}$ ($n = 15$).

values of the sun-exposed and shaded sides of fruit displayed the same trend. All the cultivars underwent changes in ethylene production linked to climacteric ripening during the 27 d at 20 °C. Ethylene biosynthesis showed a sharp rise and reached a maximum, followed by a more or less pronounced decrease at the onset of fruit senescence (Fig. 2). These events are accompanied by

the progressive decline in the I_{AD} . On the contrary, 1-MCP-treated apples showed no marked reduction in the I_{AD} values during the shelf life period, with an average decrease of 0.32 (‘Red Delicious’), 0.28 (‘Golden Delicious’), and 0.18 (‘Gala’) within 27 d. Moreover, treated fruit did not produce noticeable amounts of ethylene for most of the shelf life period.

Figure 3 shows the I_{AD} values recorded daily throughout the shelf life period plotted to the corresponding amount of ethylene biosynthesis measured each day. Each point represents the data determined for one specific day of the shelf life period. Considering the change in ethylene production in relation to the daily I_{AD} , it is possible to identify a period characterized by a range of I_{AD} values [Fig. 3 (between the vertical lines)], when the production of ethylene was higher and corresponded to the central phase of the climacteric. The identified thresholds coincide with the fruit I_{AD} levels, which displayed the maximal rate of increase/decrease in ethylene production; although after 27 d, ethylene production was still fairly high in ‘Morgenduft’ and ‘Golden’ in the first year. Threshold values were markedly different depending on cultivar. Relatively high values were recorded for ‘Morgenduft’ (1.60 and 0.62) and ‘Red Delicious’ (1.52 and 0.70, first season; 1.56 and 0.68, second season); intermediate values were recorded for ‘Golden Delicious’ (0.84 and 0.17, first season; 0.86 and 0.20, second season); and low values were determined in ‘Gala’ (0.70 and 0.18). Moreover, these threshold values associated with the highest rates of ethylene biosynthesis increase and decrease vary in different years more or less noticeably, depending on the cultivar and/or on the batch of samples considered (Fig. 3). The trends in ethylene production of ‘Red Delicious’ and ‘Golden Delicious’ were different in the 2 years. The increase/decrease in ethylene evolution was slower in the first year compared with the second. ‘Golden Delicious’ ethylene production values at 0.2 I_{AD} were much lower in year 2 than in year 1. In fact, the maximal decreases in ethylene production of ‘Golden Delicious’ were 18.25 $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ (year 1) and 42.59 $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ (year 2). Nevertheless, these decreases were measured at I_{AD} values of 0.17 (year 1) and of 0.20 (year 2), i.e., the threshold values did not differ markedly.

CORRELATION BETWEEN I_{AD} AND QUALITY PARAMETERS, PREDICTIVE MODELS. Descriptive statistics and the statistics related to the LR models obtained by I_{AD} for the analyzed parameters (SSC, firmness, and TA) for ‘Golden Delicious’, ‘Red

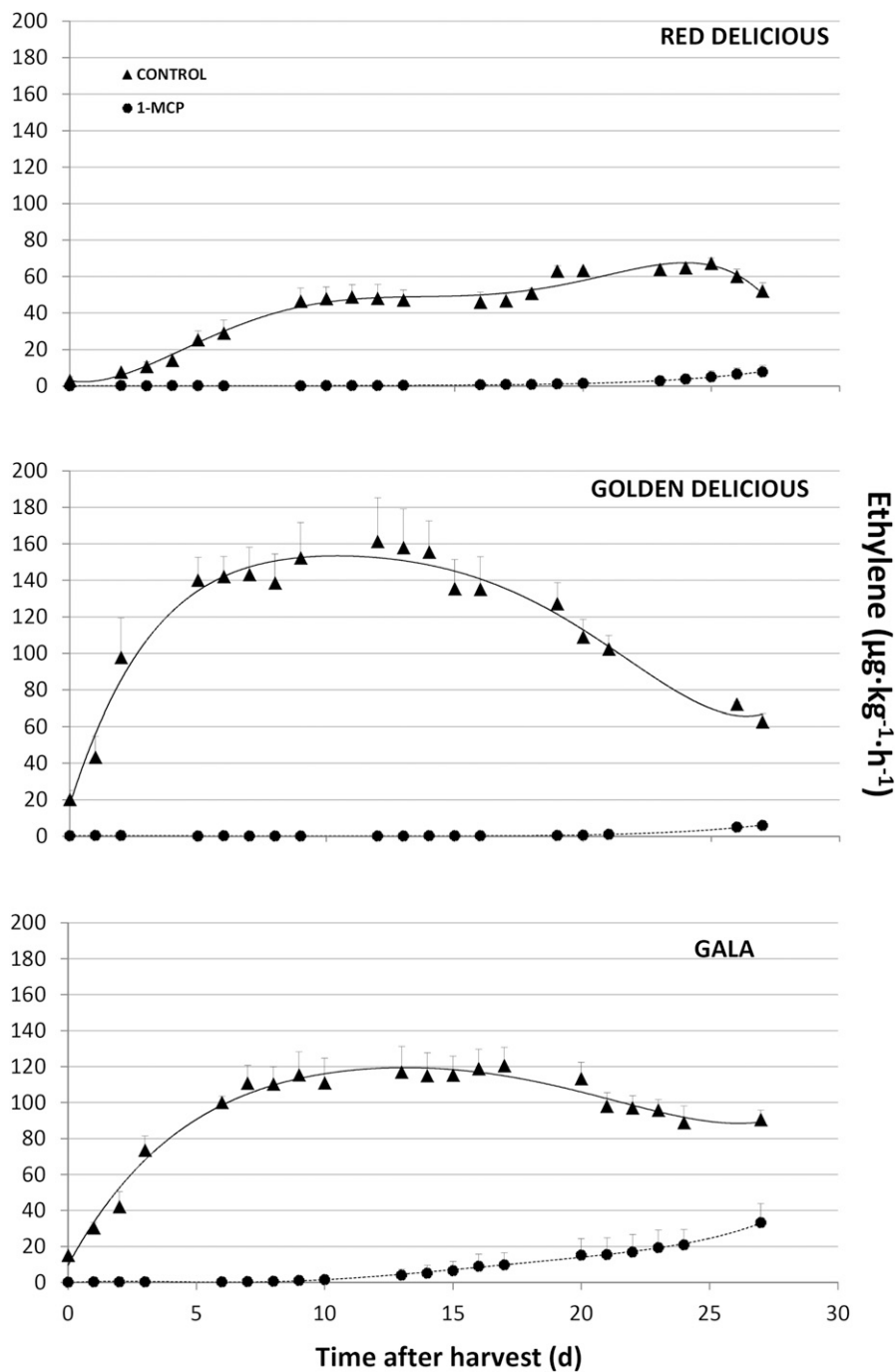


Fig. 2. Ethylene production during apple shelf life at 20 °C (68.0 °F). ‘Red Delicious’, ‘Golden Delicious’ and ‘Gala’ were treated with 1-methylcyclopropene (1-MCP) at 1 µL·L⁻¹ (ppm) for 12 h at 20 °C or left untreated. Vertical bars represent ±SE (n = 15); 1 µg·kg⁻¹ = 1 ppb.

Delicious’ and the two cultivars analyzed together are shown in Table 1. Average data are shown, considering all the sampling times. No predictive model was calculated for ‘Gala’, because I_{AD} data displayed a limited distribution and low variability.

The models developed for SSC and firmness show determination coefficients (R^2) ranging in cross-validation

from 0.60 to 0.71 and 0.65 to 0.70, respectively. Moreover, the values of RPD were slightly less than 2 (1.59 to 1.88 for SSC and 1.69 to 1.84 for firmness). The best model was developed for SSC prediction for ‘Red Delicious’ apple (RPD = 1.88) and for firmness evaluation in ‘Golden Delicious’ apple (RPD = 1.84). Results regarding models elaborated

using a combined data set for the two cultivars showed RPD values in validation for SSC and firmness of 1.59 and 1.80, respectively. Regarding TA, the best model was achieved for the ‘Red Delicious’ apple with RPD = 1.68.

I_{AD} AND QUALITY MEASUREMENTS BEFORE AND AFTER COLD STORAGE. Apples often undergo a long-term storage before reaching the market. Appropriate storage conditions imply the use of CA and often 1-MCP treatments (Smart Fresh®). Therefore quality parameters such as firmness, SSC, and TA for ‘Red Delicious’, ‘Golden Delicious’, and ‘Gala’ were determined the same day of harvest, i. e., immediately before and after a storage period. The apples harvested in orchards spread across the Valtellina region were cold-stored in warehouse storage rooms, where the fruit were maintained in air (defined in this study, as the suboptimal storage condition) or CA (the routine storage condition applied in the warehouse) using Smart Fresh® technology. Figure 4 shows the results of the trial performed in the second year. The data obtained for ‘Red Delicious’ and ‘Golden Delicious’ did not differ from the data obtained for year 1.

At harvest, the I_{AD} for ‘Red Delicious’ was 1.40 and did not change during CA storage. On the other hand, after storage in air the index decrease by ≈50%, reaching a value of 0.74. Firmness did not vary in cold-stored fruit and was 67 N both in samples at harvest and in CA, 59 N in samples stored in air. At harvest, the SSC was 12.8% and was similar after CA storage, while after storage in air it decreased to 11.4%. The level of TA differed among apples sampled at harvest and after the two storage conditions. The range was from 4.07 g·L⁻¹ at harvest to 1.61 g·L⁻¹ after air storage.

Similar I_{AD} values (0.96 and 1.08) were registered at harvest and after CA storage in ‘Golden Delicious’. There was a drop of about 33% in the I_{AD} values of samples maintained in air. No changes in firmness were recordable during cold storage in CA. Fruit analyzed before and after CA storage had firmness of 66 and 70 N, respectively. When stored under air, fruit softened significantly and firmness decreased by 22

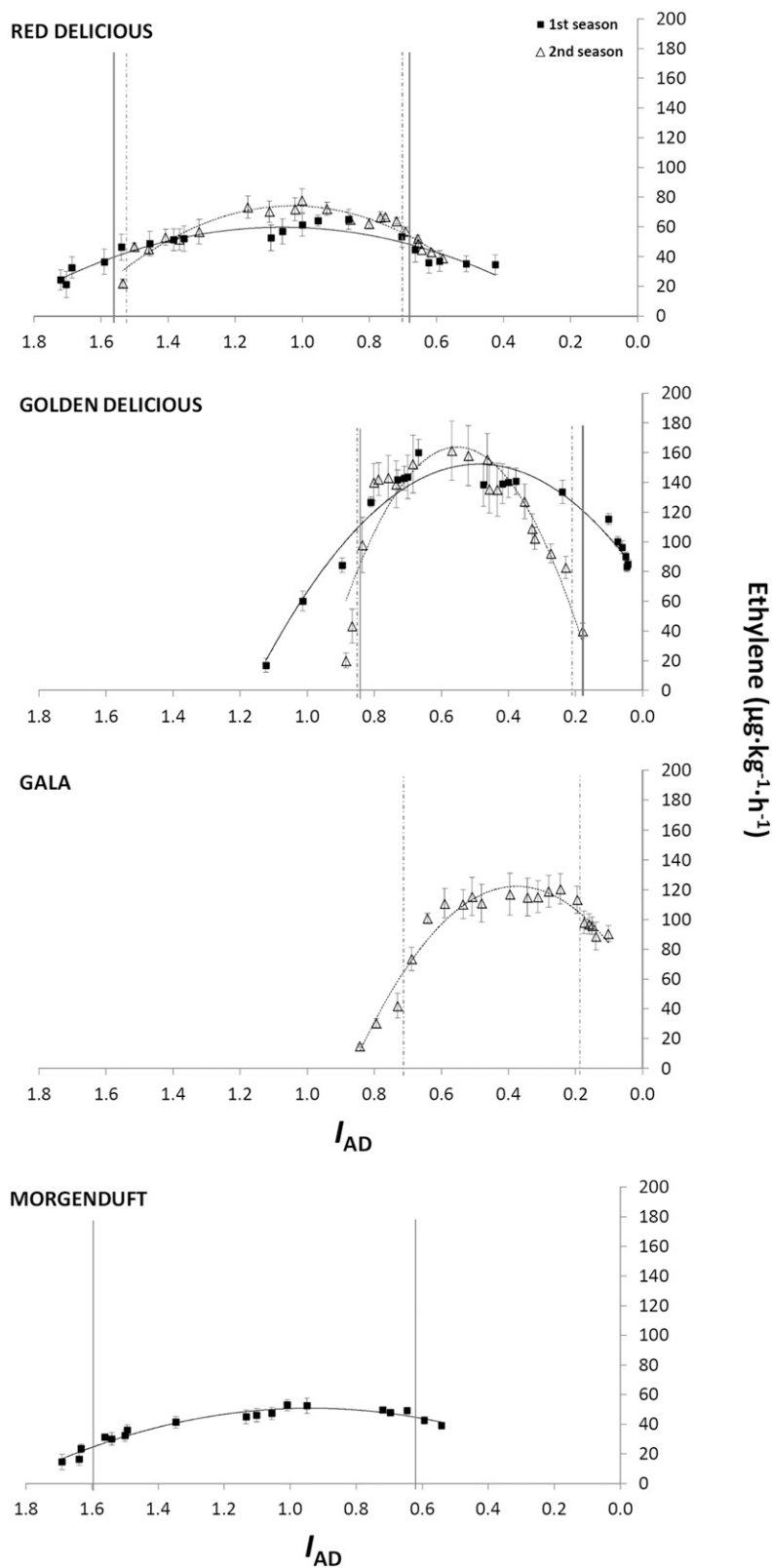


Fig. 3. Ethylene production as a function of the index of absorbance difference (I_{AD}) during shelf life at 20 °C (68.0 °F) of ‘Red Delicious’, ‘Golden Delicious’, ‘Morgenduft’ and ‘Gala’ apples. Solid (first year of trial) and dashed (second year of trial) lines indicate the I_{AD} ranges linked to the central phase of climacteric. Vertical bars represent \pm SE ($n = 15$); $1 \mu\text{g}\cdot\text{kg}^{-1} = 1 \text{ ppb}$.

N. No significant differences in SSC were observed among different samples; values ranged from 14.6% to 13.3%. TA followed a pattern like that for I_{AD} and firmness, and there were no changes after CA storage. However, after air storage TA values approximatively halved.

‘Gala’ had the lowest I_{AD} values of the cultivars studied, 0.36 at harvest and 0.50 and 0.27 in CA and air stored apples, respectively. No differences in I_{AD} were recorded in ‘Gala’ fruit at harvest or after different storage regimes. No changes in firmness after CA storage were identified, whereas firmness was 34% lower in fruit stored under air with respect to harvest time. ‘Gala’ had a SSC of 12.8% at harvest; this increased during CA storage, reaching 14.4%; SSC after air storage (13.4%) did not differ from the values at harvest or after CA storage. TA did not change during cold storage and ranged from 3.4 to 2.5 $\text{g}\cdot\text{L}^{-1}$.

Discussion

The I_{AD} values at harvest differed among the four cultivars, showing different slopes for I_{AD} progressive decrease. In fact, chlorophyll concentration and metabolism, i.e., its synthesis, breakdown and adaptation to environmental changes, varies depending on genetic background (Merzlyak et al., 2002). ‘Gala’ fruit skin had low I_{AD} values at harvest and that approached zero before the end of the shelf life period. Indeed, McGlone et al. (2002) reported in ‘Gala’ a very dramatic reduction of the chlorophyll absorbance peak during the course of the harvesting period. The results showed that a single I_{AD} value cannot be regarded as a harvest index for different cultivars, as assessed also for other species (Infante et al., 2011a). The I_{AD} measured on the exposed side of the fruit skin, i.e., the skin with the blush color, of ‘Red Delicious’, ‘Morgenduft’, and ‘Gala’ was always higher, regarding each single fruit, than values for the shaded side. However, differences between daily averages on the two fruit sides were not statistically significant, except for ‘Gala’. On the contrary, in the yellow ‘Golden Delicious’ the higher values were recorded on the shaded sides of each fruit. Our results are in agreement with those of other authors who reported that exposed

Table 1. Descriptive statistics and statistics of the linear regression (LR) models elaborated on DA meter data Index of absorbance difference I_{AD} to estimate qualitative parameters of ‘Golden Delicious’ and ‘Red Delicious’ apples. Data of samples before (i.e., immediately after harvest) and after storage in controlled atmosphere (CA) of both years of trial were used.

| Cultivar | Quality parameters | Mean | SD | n | Calibration | | | Cross-validation | | |
|-------------------------------------|---|-------|-------|-----|-------------|--------------------|-------|---------------------------------|-------------------------------------|------------------|
| | | | | | R^2 | RMSEC ^y | R^2 | RMSEC ^v ^y | RMSEC ^v (%) ^y | RPD ^y |
| Golden Delicious | SSC (%) ^z | 13.60 | 0.93 | 60 | 0.65 | 0.86 | 0.60 | 0.93 | 6.84 | 1.60 |
| | Firmness (N) | 55.77 | 10.41 | 60 | 0.72 | 5.40 | 0.70 | 5.65 | 10.13 | 1.84 |
| | TA [malic acid (g·L ⁻¹)] ^z | 3.12 | 0.71 | 30 | 0.54 | 0.47 | 0.41 | 0.53 | 16.99 | 1.35 |
| Red Delicious | SSC (%) ^z | 12.99 | 1.95 | 100 | 0.72 | 1.02 | 0.71 | 1.04 | 8.01 | 1.88 |
| | Firmness (N) | 61.43 | 8.25 | 100 | 0.66 | 4.82 | 0.65 | 4.89 | 7.96 | 1.69 |
| | TA [malic acid (g·L ⁻¹)] ^z | 2.23 | 0.73 | 50 | 0.68 | 0.41 | 0.64 | 0.44 | 19.73 | 1.68 |
| Golden Delicious + Red Delicious | SSC (%) ^z | 13.14 | 1.86 | 160 | 0.61 | 1.16 | 0.60 | 1.17 | 8.90 | 1.59 |
| | Firmness (N) | 60.31 | 8.98 | 160 | 0.69 | 4.95 | 0.69 | 5.00 | 8.29 | 1.80 |
| | TA [malic acid (g·L ⁻¹)] ^z | 2.51 | 0.83 | 80 | 0.22 | 0.73 | 0.17 | 0.75 | 29.88 | 1.11 |

^zSSC = soluble solids concentration; TA = titratable acidity.

^yRMSEC = root mean standard error of calibration, RMSEC^v = root mean standard error of cross-validation, RPD = ratio performance deviation.

1 N = 0.2248 lbf; 1 g·L⁻¹ = 1000 ppm.

and shaded peels of both red and green-colored apple cultivars differ in their chlorophyll concentration (Kuckenberget al., 2008; Merzlyak et al., 2002). In fact, anthocyanin pigments can be considered as a factor protecting chlorophyll from photo-destruction due to their lightscreening effects and their function against light-induced stress (Merzlyak and Chivkunova, 2000). Moreover, a higher level of chlorophyll in exposed compared with shaded skin of red cultivars might be necessary to maintain an optimal rate of photosynthesis under the shielding layer of anthocyanins (Merzlyak et al., 2002).

Since apple is a climacteric fruit, ethylene plays a major role in the ripening process. For this reason, changes in I_{AD} occurring during the shelf life were related to the time course of ethylene production which, in turn, characterizes the progression of fruit ripening. The I_{AD} changes in relation to ethylene production allowed us to identify characteristic stages of fruit ripening and senescence for each cultivar tested, within distinctive I_{AD} ranges. These ranges did not change greatly over different years and were quite stable, although the trend in ethylene production was different in the 2 years. The increase/decrease in ethylene evolution was slower in the first year compared with the second and ‘Golden Delicious’ ethylene production values at the 0.2 I_{AD} were much lower the second year, compared with the first. Nevertheless, the I_{AD} values that marked the beginning and the end of the central phase of the climacteric, i.e.,

the maximal rate of increase/decrease in ‘Red Delicious’ and ‘Golden Delicious’ were similar in both years, and only few variations were recorded. In fact, orchard, region and seasonal variation can affect both chlorophyll levels and rate of the ripening process in different ways, also depending on the genetic background of the fruit. When apples were treated with 1-MCP, the consistent drop in I_{AD} values during shelf life was greatly reduced in all cultivars, indicating a slower skin yellowing, i.e., a lower chlorophyll decrease as ethylene production was greatly inhibited and delayed. Effects of 1-MCP treatment actually include, along with reduced ethylene production and respiration, delays in fruit degreening and softening (Baritelle et al., 2001).

Predictive models were developed to assess the I_{AD} as a tool for apple quality evaluation. The predictive values of I_{AD} for firmness, SSC, and TA were evaluated separately for the red cultivar Red Delicious and the yellow Golden Delicious and then values were grouped together. Samples of both years of the trial were used for the models. Predictive models based on the grouped set gave similar results to the models based on any cultivar taken individually. Results were comparable in terms of R^2 and RMSE for quality attributes of firmness and SSC. Nyasordzi et al. (2013) found better correlations taking into account the grouped set instead of any cultivar taken by itself. The predictive models for TA showed higher R^2 and lower percent RMSEC^v for the single cultivars,

due probably to different patterns (and different slopes) of TA evolution in relation to I_{AD} changes, i.e., to different patterns of organic acid metabolism in relation to chlorophyll degradation during postharvest life. These results slightly disagree with those obtained by Nyasordzi et al. (2013), who obtained better correlations of I_{AD} for SSC and TA than for firmness. Indeed, firmness is a parameter linked to fruit softening, a complex process that results from multiple flesh tissue changes, related both to cell wall disassembly and reduction of turgor pressure. It has not been well clarified yet how these changes are related to the structural and mechanical attributes of fruit. Moreover, other studies report poor prediction models for fruit texture based on the analysis of spectral properties using optical techniques (Lu et al., 2000; Peirs et al., 2002; Zude et al., 2006). Models elaborated by authors for firmness prediction showed encouraging results, with similar RMSE and slightly worse R^2 (0.7 vs 0.8), compared with those published by Fan et al. (2009). Fan et al. achieved their results by a more complicated application based on Vis/NIR transmittance. Lu (2007) used hyperspectral scattering imaging on ‘Golden Delicious’ and ‘Red Delicious’ apples, in the spectral region between 500 and 1000 nm. Neural network models were built to predict fruit firmness obtaining, with an external validation set, standard errors of prediction of 6.2 N for ‘Golden Delicious’ and 6.1 N for ‘Red Delicious’. The application of NIR spectroscopy for the analysis

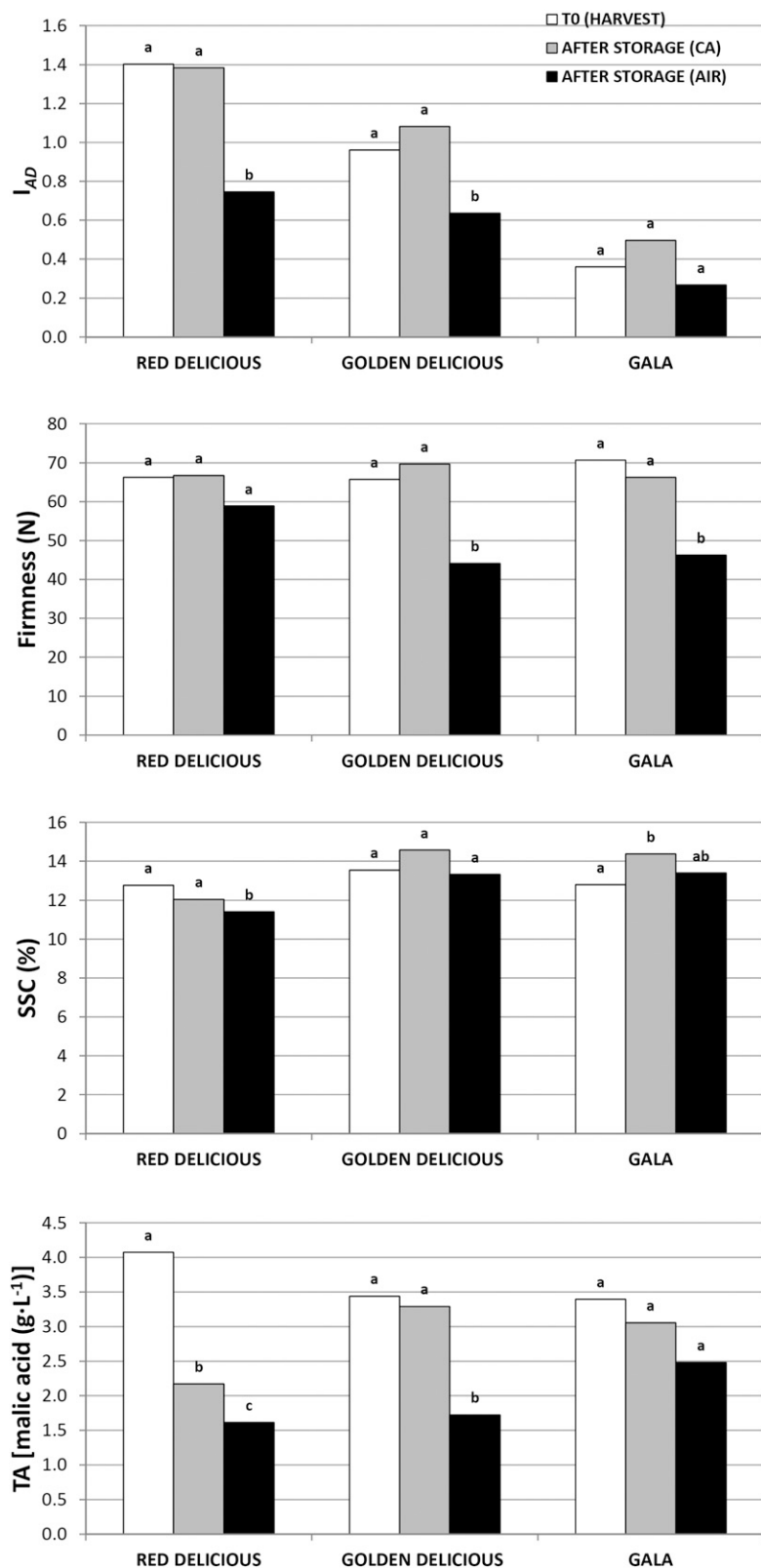


Fig. 4. Quality attributes [i.e., index of absorbance difference (I_{AD}), firmness, soluble solids concentration (SSC), and titratable acidity (TA)] of ‘Red Delicious’, ‘Golden Delicious’, and ‘Gala’ apples before and after different storage regimes. Apples were stored under air or under controlled atmosphere (CA) [1% oxygen, 2.5% to 3% carbon dioxide, 94% to 99% relative humidity, and 1 to 1.5 °C (33.80 to 34.70 °F)], using Smart Fresh® (Agro-Fresh, Philadelphia, PA) technology [1-methylcyclopropene (1-MCP)]. Columns with different letters are significantly different at $P = 0.05$. ($n = 15$ for ‘Golden Delicious’, $n = 25$ for ‘Red Delicious’ and ‘Gala’); $1 \text{ N} = 0.2248 \text{ lbf}$, $1 \text{ g}\cdot\text{L}^{-1} = 1000 \text{ ppm}$.

of this parameter often encountered considerable difficulties, highlighted by published studies (Nicolai et al., 2008). Nevertheless, some authors found correlations of fruit firmness with chlorophyll concentrations (Infante et al., 2011b; Kuckenberg et al., 2008). Nyasordzi et al. (2013) reported an RMSE of 10% and a R^2 of 0.51 for the correlation between I_{AD} and firmness at harvest. The correlation coefficients of our predictive model for firmness are fairly promising and, although the RPD of the comprehensive model was little less than 2, a RMSECV value of 5 is encouraging. In fact, Harker et al. (2002a) reported that apple flesh firmness needs to differ by a minimum of 6–8 N before a difference in sensory texture attributes can be perceived by panelists. Predictive models for SSC were also fairly good in terms of R^2 and percent RMSECV, similar to those reported by Nyasordzi et al. (2013). Reports obtained for different fruits by different authors using other optical techniques (e.g., NIR spectroscopy) showed values of RMSECV about 0.6% to 1% (Bobelyn et al., 2010; Nicolai et al., 2007). In our work the RMSECV values were about 1%, while differences between apples of more than 1% are required before trained panelists can detect a difference in sweet taste (Harker et al., 2002b). Conversely, TA data were not adequate for development of predictive models between I_{AD} and TA. Models were poor in prediction terms, with the highest R^2 (0.64) obtained for ‘Red Delicious’. Analysis of the data of ‘Red Delicious’ and ‘Golden Delicious’ together did not improve the predictive model, even though a greater number of samples had been taken into account. This may be because of differing cultivar metabolic processes, as mentioned above. Predictive models showed encouraging results and suggest the possibility to use the DA meter for “in line” processes like sorting and real-time classification of fruits in homogeneous lots based on the non-destructive evaluation of ripening/quality indices.

Comparisons between different storage conditions confirmed that I_{AD} is a promising tool for evaluating the quality of apples after the storage period. Apples stored in optimal conditions, viz under CA and using Smart

Fresh® technology, showed no changes both in I_{AD} and in quality indices. Conversely, after a storage period under air, I_{AD} values for ‘Red Delicious’ and ‘Golden Delicious’ were significantly lower, suggesting an overripe or senescent phase of fruit and a lower overall quality. ‘Gala’ did not show a significant decrease, but the weakness in I_{AD} prediction may be due to the narrow I_{AD} distribution of the data set. Toivonen and Hampson (2014) reported for 1-MCP treated ‘Royal Gala’ no clear association during cold storage between I_{AD} values and internal ethylene concentration, firmness, and TA. These authors analyzed the effects of 1-MCP and two different CAs on changes in I_{AD} values and internal quality attributes. They concluded that different apple cultivars showed differing responses with regard to the relationship of I_{AD} changes to quality attributes, after 1-MCP treatment and CA storage. Furthermore, they ascertained that I_{AD} did not consistently correlate to attributes other than chlorophyll *a* concentration. In our study, stored apple quality indices followed the same pattern of I_{AD} , although the differences were not always significant. This was more evident for SSC levels. An explanation for the different evolution of this parameter might be a residual conversion of the starch to soluble sugars during storage and a subsequent increase of SSC levels, partially balanced with a higher oxidative metabolism in air. I_{AD} depends on chlorophyll concentration and consequently on ripening stage, hence it is not directly linked to the constituents or properties of quality attributes. That is, predictive models for quality indices are linked to the chlorophyll reduction as fruit ripen and they do not directly reflect soluble solids or organic acids concentrations or cell wall disassembly at all. Therefore, there might be differences due to different genetic backgrounds in SSC, TA, and firmness evolution during ripening that are not always related to chlorophyll degradation. Moreover, different cultural conditions, different growing regions and seasonal variations could affect fruit chlorophyll levels (McGlone et al., 2002; Reay et al., 1998) with concomitant effects on I_{AD} values and the maturation process, but in distinctly different ways.

For instance, preharvest factors such as treatments with plant growth regulators may interfere with the interpretation of I_{AD} values, dissociating these values from other quality/maturity parameters (Doerflinger et al., 2016). There is correlation between I_{AD} and quality/maturity indices as long as these indices change in a coordinated manner with the decrease in I_{AD} values.

As reported by Johnston et al. (2009), different fruit ripening characteristics and quality attributes vary both in ethylene sensitivity and dependency. The response curves to ethylene may shift for different cultivars and for fruit exposed to different conditions not only during growth, but also throughout the postharvest life, as in the case with 1-MCP treatments or CA storage. This could lead to different patterns of correlation between chlorophyll concentration (reflected by I_{AD} values) and other quality attributes. Moreover, it is not always possible to include more cultivars in one predictive model, as in the case of TA of Red Delicious and Golden Delicious. That is, correlations of I_{AD} values with harvest indices and other quality attributes may depend on cultivar. Nevertheless, the results before and after storage were obtained from apples harvested in different orchards, seasons and microclimatic conditions, so the experimental approach was characterized by a great heterogeneity of samples. In these conditions, I_{AD} evaluation provided a good marker of storage quality.

Concluding remarks

On the basis of the results obtained during the shelf life period of apples and the predictive models developed for quality attributes, the DA meter can be regarded as a promising tool, not only for evaluating the ripening stage of apples, but also for monitoring postharvest quality attributes. It could help operators in planning the distribution sequence and enable retailers to identify and sort homogenous fruit batches with similar characteristics. The commercial handling of apples is characterized by the need for quick measurements on heterogeneous fruit material to obtain an efficient classification of lots. In this study, the use of the DA meter provided fairly good prediction models of quality/ripening indices,

thus confirming the reliability of this tool to be used on a commercial scale by warehouses and large-scale retailers.

This device can therefore be used as a nondestructive tool in real industrial conditions for apple sorting into lots. This would allow better management of the fruits during the storage period and reduce product wastage. In fact, the use of this tool could help to classify apples by ripening stage and thus, “more ripe” fruit could be destined for a shorter storage period, while “less ripe” fruit could be kept in cold rooms for a longer period without quality losses.

Moreover, use of DA meter would permit a quick monitoring of important ripening/quality parameters both at harvest and during all stages of storage and a better planning of the removal from storage based on fruit characteristics. Nevertheless, I_{AD} cannot be seen as an overall replacement of standard ripening indices, but it can effectively supplement data from these parameters. Further investigations are needed to evaluate other apple cultivars and to extend the determinations to more seasons and across different growing regions, to obtain more robust predictive models.

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