agronomy

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A Study on the Potential of IAD as a Surrogate Index of Quality and Storability in cv. 'Gala' Apple Fruit

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Received: 6 September 2019; Accepted: 8 October 2019; Published: 16 October 2019

Abstract: The decline of relative chlorophyll contents during fruit ripening is considered to be an important indicator of fruit physiological condition. The recent availability of low-cost portable visible spectrum (VIS) spectrometers has spurred research interest towards optical sensing of chlorophyll changes in intact fruit, with many scientists attempting to link the shifts in optical signals, attributed to chlorophyll changes, to different maturity and quality parameters. One of the widely available portable devices for non-destructive estimation of relative chlorophyll contents is the DA meter, which provides a maturity index that is calculated as a difference between absorption at 670 nm (near the chlorophyll-a absorption peak) and 720 nm (background of the spectrum), abbreviated as IAD. In the present study, the evolution of IAD and its relation to starch pattern index (SPI) and fruit flesh firmness (FFF) was monitored in fruit of two cv. 'Gala' clones during maturation and storage, aiming to identify a potential existence of a usable IAD range for the assessment and prediction of the optimal harvest window and storage potential. In both clones, canopy positions, fruit sides, and seasons IAD, SPI, and FFF generally changed in a linear fashion over time, but with partially very different slopes, i.e., they were changing at different rates. What all of these parameters had in common was the presence of a very high biological variability, which is typical of apple fruit. Significantly powerful estimations of SPI ($r^2 > 0.7$, $p < 0.005$) and pre- and post-storage FFF ($r^2 > 0.6$, $p < 0.005$) were achieved. However, the very large biological variability could not be neutralized, which means that the predictions always included large confidence intervals of up to 0.46–0.59 units for SPI and 0.82–1.1 kgF FFF, which ultimately makes them unusable for practical applications. Experiments done under real-life conditions in a commercial fruit storage facility on several different fruit batches confirmed that IAD measured at harvest cannot be used indiscriminately for predicting post-storage FFF of cv. 'Gala' originating from different orchards. Nevertheless, mean IAD values that were obtained at optimal maturity from samples of the same orchards remained stable over seasons (0.8–1.2), which strongly suggests that, provided that the calibrations and validations are not only cultivar, but also orchard-specific, IAD has a potential for estimating maturity and storability of apple fruit. In this case, IAD could replace standard maturity indices, otherwise it would be suited for use as a supplementary index for determining fruits physiological maturity status.

Keywords: non-destructive methods; spectrometry; chlorophyll index; maturity; firmness; *Malus x domestica*

1. Introduction

The precise determination and prediction of fruits optimal physiological stage for harvest, related to appropriate postharvest strategies, is crucial for supplying consumers with fruit of high quality, even after prolonged storage and shelf-life. A long-established practice of determining the optimal harvest window in the apple industry is to combine several physiochemical parameters, like starch pattern index (SPI), Magness-Taylor fruit flesh firmness (FFF), and soluble solids content (SSC) and express them as different maturity indexes. While these methods are simple and inexpensive, they are based on destructive analyses, with the obtained data presenting low repeatability and high vulnerability to interpretation errors [1,2].

In recent years, various methods for quality and ripeness evaluation of intact agricultural products have been developed [3–7]. A potential technique for assessing fruits physiological stage is to monitor changes in its spectral signature within the visible light spectrum, especially in the range that was governed by chlorophyll fluorescence or absorption [8–12]. During ripening, the fully functional photosynthetic apparatus of the apple fruit [13] disassembles, which involves chloro-to-chromoplast conversions, which results in a gradual breakdown of chlorophyll [14–16]. Thus, the decrease of relative chlorophyll content is an important index of fruit physiological characteristics and its content, determined either destructively or non-destructively, has been considered for estimating the optimal harvest window as well as the quality of apple fruit [2,3,6,8,16–22].

A portable, optical device, the DA-meter, can non-invasively assess the relative chlorophyll levels in apple fruit (Sinteleia, Italy). The reflectance spectrum resulting from light interaction with the skin and pulp measured by the instrument is converted to absorbance based on Lambert Beer's Law [23]. Subsequently, an index of relative chlorophyll content (IAD) is calculated as a difference between absorption at 670 nm (near the chlorophyll-a absorption peak) and 720 nm (background of the spectrum), abbreviated as IAD [23].

IAD has been found to strongly correlate to total chlorophyll [24], chlorophyll-a [25], and to Normalized Difference Vegetation Index (NDVI), which in turn was found to have strong correlations with chlorophyll-a contents [26,27] in different apple cultivars. Toivonen and Hampson [25] found strong correlations ($r^2 > 0.9$) between IAD and SPI in cv. 'Ambrosia' apples during ripening. IAD was also strongly correlated to SPI ($r^2 = 0.9$), with moderate correlations being observed for FFF ($r^2 = 0.7$) in cv. 'Gala' apples grown in Piedmont [28]. However, in the study done in the same region in another season, IAD did not show any significant correlations with FFF and SPI [29]. Moderately strong correlations, with a magnitude above 0.7, were also observed between IAD, FFF, and SPI for cv. 'Starking' apples [30]. On the other hand, weak correlations ($r^2 < 0.2$) between IAD with SPI and FFF were recorded for cv. 'Empire' apples [31], with poor correlations ($r < 0.5$) observed between IAD and FFF for cv. 'Braeburn', 'Cripps Pink' [20], and cv. 'Golden Delicious' apples, whereas relevant correlations were obtained in cv. 'Gala' [32]. A conclusion that was drawn from the results of the aforementioned studies is that the relations found between IAD and FFF, SPI, or other quality parameters are not only cultivar- and season-, but also region specific. Thus, as is often the case with non-destructive methods, the applicability of the IAD should be tested and validated not only at genotype, but also at seasonal and regional levels.

The presented results are an attempt to evaluate the potential of the spectral chlorophyll index IAD for assessing maturity and predicting postharvest quality at the time of harvest of apple fruit. This study was conducted both under controlled test and real-life conditions on cv. 'Gala', a commercially important apple cultivar worldwide, as former reports indicated it as being highly responsive to IAD. To this aim, the evolution of IAD was monitored by means of a portable spectrometer (DA Meter, Sinteleia, Italy) during maturation and after regular atmosphere storage (RA) of two cv. 'Gala' clones that were grown in the same orchard, at different fruit and canopy positions, over two growing seasons and set in relation to the evolution of SPI and FFF.

2. Materials and Methods

2.1. Plant Material

The study was carried out on apple fruit (*Malus x domestica* Borkh.) of two cv. 'Gala' clones (Schniga and Buckeye) from fully mature trees of uniform vigor and crop loads, grown in a "Good Agricultural Practice"(GAP)-certified orchard near Bolzano (South Tyrol, Italy) at an elevation of 220 m a.s.l. Based on the respective goals and objectives, several experiments with varied numbers of fruit per analysis were conducted (see Sections 2.2–2.4).

In the experiments that were performed at a commercial apple storage facility, a mix of different cv. 'Gala' clones from different producers, but of comparable maturity were used, as described in more detail in Section 2.5.

2.2. Methods for Determining Relative Amounts of Chlorophyll, Starch Pattern Index, Flesh Firmness and Optimal Maturity Stage

All of the destructive and non-destructive parameters were assessed in the laboratory under ambient light and temperature conditions.

Starch pattern index (SPI) of iodine-stained apple discs was visually assessed by means of the Laimburg five-point scale, used for commercial assessments of starch degradation in South Tyrol (Italy). In this scale, the value 1 represents the maximum and value 5 represents the minimum possible iodine staining.

Fruit flesh firmness FFF (kgF) was analyzed along with other quality parameters with an automated analyzer "Pimprenelle" (Setop Giraud Technologie, Cavaillon, F). Pimprenelle consists of three measuring units: a penetrometer, an optical refractometer, and a pH-titrator. This enables the simultaneous analysis of different quality parameters [33].

Non-destructive measurements were conducted with a portable commercially available and patented instrument "DA meter" (Sinteleia, Italy). "DA-meter" consists of three LEDs emitting radiation at 670 nm and three LEDs emitting radiation at 720 nm, which are positioned circularly around the photodiode sensors. The spectra that were measured by the instrument are converted to absorbance based on Lambert Beer's Law [23]. The output of the instrument is the IAD, and index of relative chlorophyll content, calculated as a difference between absorption at 670 nm (near the chlorophyll-a absorption peak) and 720 nm (background of the spectrum).

The optimal harvest date was determined based on local recommendations (SPI 2.5–3 on a five-point scale, FFF 7.5–8 kgF), when mainly considering SPI and it corresponded to 13 August 2015, and 10 August 2017.

2.3. Pre-Harvest Study

For the purpose of monitoring changes of IAD, SPI, and FFF during fruit ripening with a view of pinpointing potential maturity-specific IAD ranges, pre-harvest evaluations were performed in two growing seasons. In 2015, 10 inner-canopy and 10 outer-canopy fruit per clone were picked in three repetitions at every assessment, carried out eight times, twice weekly, from 6 to 31 August 2015. On these fruits, SPI, FFF, and IAD were assessed, with the IAD measurements being taken from the sun-exposed fruit side.

In 2017, 10 fruit per clone were taken for measurements in three repetitions, with no distinction between canopy positions, but with the IAD taken from both the sun-exposed and the shaded fruit side. The sampling period lasted from 31 July to 4 September 2017, with the apples being assessed eight times.

2.4. Post-Harvest Study

A storage experiment was conducted in the growing season 2015 to evaluate the predictive potential of IAD on storability and keeping quality of apple fruit. Fruit were picked four times at weekly intervals, on 06, 13, 20, and 27 August 2015, whereby attention to separate the fruit according to their position within canopy was payed. After 2.5 months of RA storage (21% O_2, 1.3 °C, 0.04% CO_2, 70% relative humidity), 30 randomly selected fruit per batch were evaluated for their IAD and FFF. Further 30 fruit per batch were evaluated after seven days of shelf life (20 °C, 70% relative humidity).

2.5. Practical Application Test

Additional tests were carried out in a commercial fruit storage facility on cv. Gala apples from the same region to test the applicability of IAD under real-life conditions. In commercial storage facilities, apple fruit of different origins and clones, albeit with similar maturity stages, are normally stored together. This was also the case in our study, in which 69 batches that were composed of different cv. 'Gala' clones from different orchards, but of comparable maturity, were stored together. At harvest, IAD measurements were done on sun-exposed and shaded fruit sides on the 1370 selected fruit from 69 batches. It was attempted to conduct the measurements while considering fruit side, but these were not always easily distinguishable. After IAD measurements, the fruit were numbered and stored under controlled atmosphere conditions (1% O_2, 2% CO_2, 1.3 °C) for six months. After storage, FFF was assessed on the sun-exposed and the shaded side of the fruit.

2.6. Statistical Analyses

Data were analyzed with descriptive statistics and inferential statistics of ANOVA (* $p < 0.005$) as well as with linear regression using SPSS and R.

3. Results and Discussion

3.1. Kinetics of IAD in Relation to Starch Pattern Index and Fruit Flesh Firmness during Maturation

In both clones, for both inner and outer canopy positions and on both fruit sides, in both seasons, a similar time-course of IAD values was observed (Figure 1, Tables A1 and A2). Within that period, mean IAD significantly decreased (Tukey HSD, $p > 0.005$) from the initial values of 1.3–1.5 to 0.3–0.4, but with a very high variability being observed between individual fruit, especially at the time of optimal maturity (13 August 2015 & 10 August 2017). Subsequently, the IAD decreased asymptotically to the lower limit of 0 (Figure 1, Tables A1 and A2). The fact that, in the current study, IAD revealed a linear trend, turning into exponential in ripe fruit is a strong indication that chlorophyll degradation in the fruit skin was already in full swing by the time the IAD measurements started. Possibly only due to the narrow time frame around the optimal harvest date chosen for monitoring, a linear pattern with very similar values was observed in 2011 for different cv. 'Gala' clones that were taken from five different orchards from the same region [32]. Similarly, a linear trend was observed in cv. 'Gala' grown in Piedmont [28]. Others observed values stabilizing at around 0.2 for the cv. 'Honeycrisp' subsequent to a linear trend [34], in agreement with the approximate exponential degradation kinetics of the spectral chlorophyll index in cv. 'Golden Delicious' during maturation [27].

Chlorophyll degradation revealed a sigmoidal pattern during apple fruit ripening [35]. Given that IAD is a measure of relative chlorophyll, it is safe to assume that it also changes in a sigmoidal fashion.

SPI revealed an inverse sigmoidal pattern as compared to IAD (Figure 2, Tables A1 and A2), which implies that the rate of chlorophyll degradation could be synchronized with the one of starch. Thus, the non-destructive monitoring of IAD could serve as a proxy for SPI-based maturity prediction of cv. 'Gala'.

Depending on the season, clone, or canopy position, in the monitored period, FFF linearly decreased from around 9–10 kgF to around 5–7 kgF (Figure 3, Tables A1 and A2). Unlike IAD, in the monitored period, FFF was not approaching its lower limit. Thus, despite both IAD and FFF theoretically changing in a sigmoidal fashion [36,37], their rate of change is apparently not synchronized. Moreover, IAD revealed a higher relative variability, as compared to FFF and SPI (Tables A1 and A2). In optimally mature apples the coefficient of variability of IAD, SPI, and FFF amounted to around 30%, 20%, and 10%, respectively.

Over maturation time, the relative variability of IAD increased, whereas it decreased in the case of SPI, while FFF revealed near constant relative variability (Figure 4, Tables A1 and A2). Similar temporal differences in the distribution of IAD, SPI, and FFF during ripening were also observed in cv. 'Gala' from Piedmont [28]. Based on these observations of heterogeneity differences, it is very likely that any potential correlations between the abovementioned parameters would be characterized by large confidence intervals.

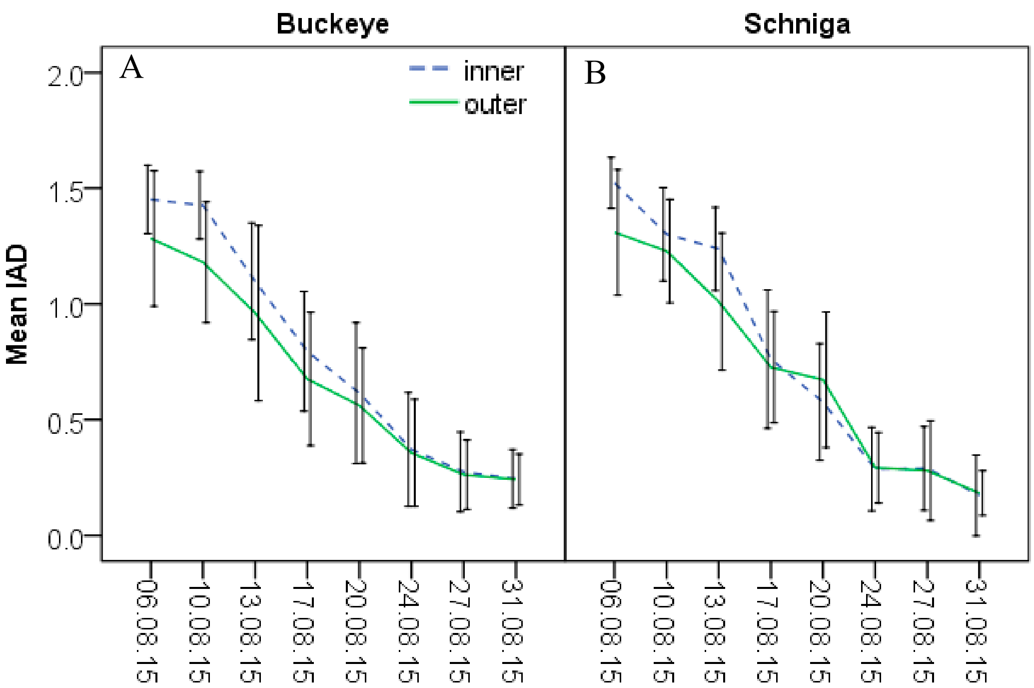


Figure 1. Changes (mean and standard deviation) of index of relative chlorophyll content (IAD) in the inner and outer canopy fruit during maturation (6 August to 31 August) in 2015 for (**A**) cv. 'Gala Buckeye' and (**B**) cv. 'Gala Schniga'.

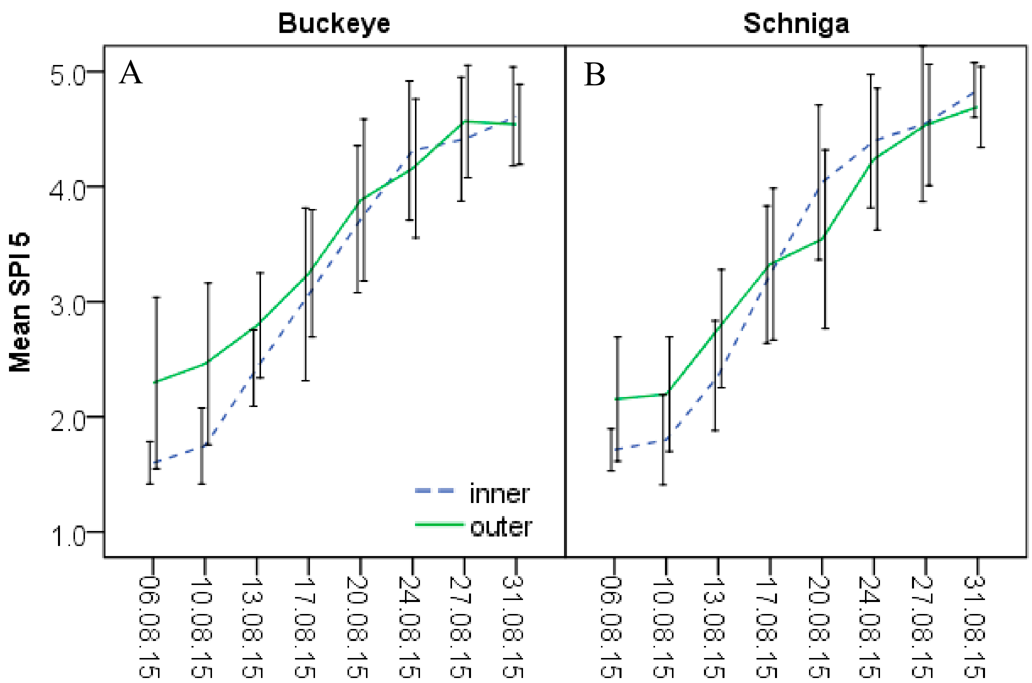


Figure 2. Changes (mean and standard deviation) of starch pattern index (SPI 5) in the inner and outer canopy fruit during maturation (6 August to 31 August) in season 2015 for (**A**) cv. 'Gala Buckeye' and (**B**) cv. 'Gala Schniga'.

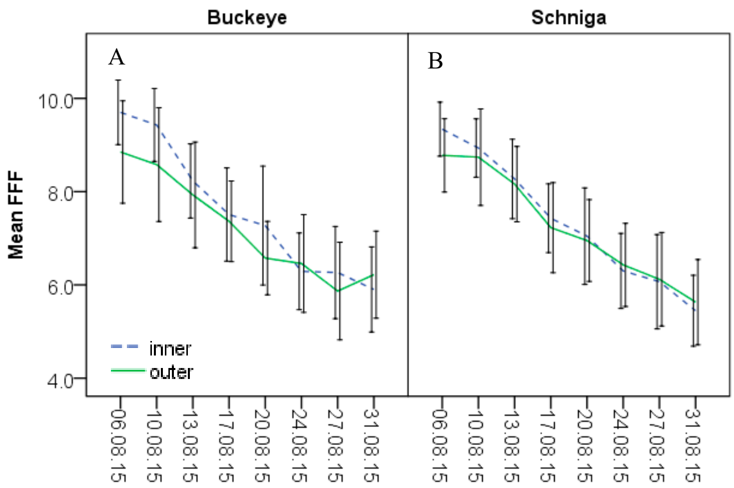


Figure 3. Changes (mean and standard deviation) of firmness (FFF) in the inner and outer canopy fruit during maturation (6 August to 31 August) in season 2015 for (**A**) cv. 'Gala Buckeye' and (**B**) cv. 'Gala Schniga'.

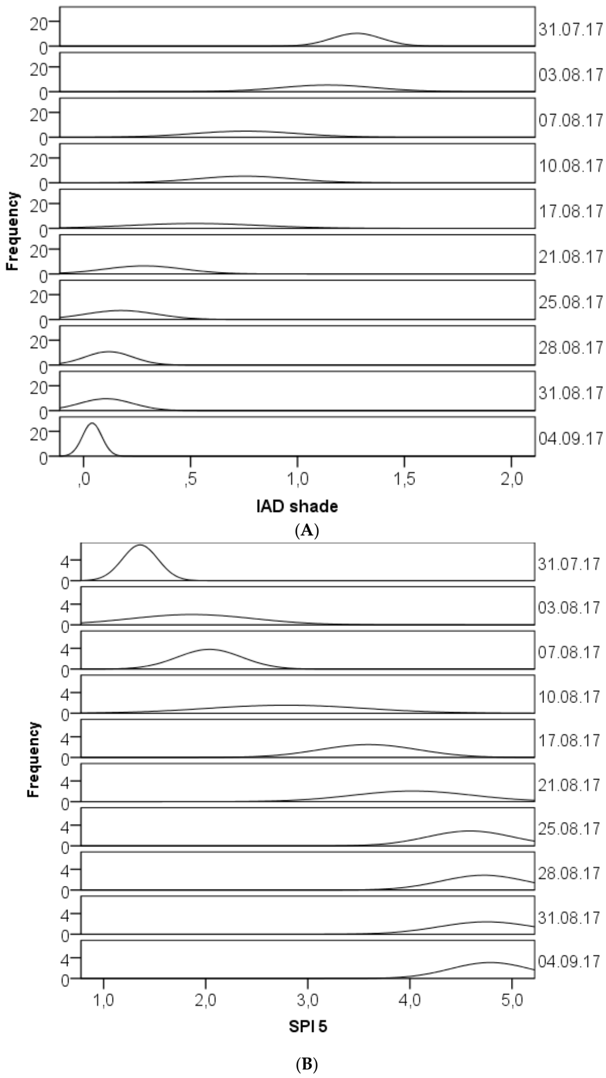
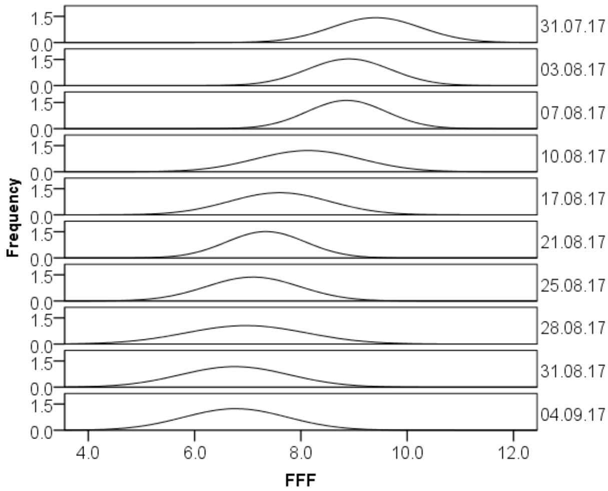


Figure 4. *Cont.*



(C)

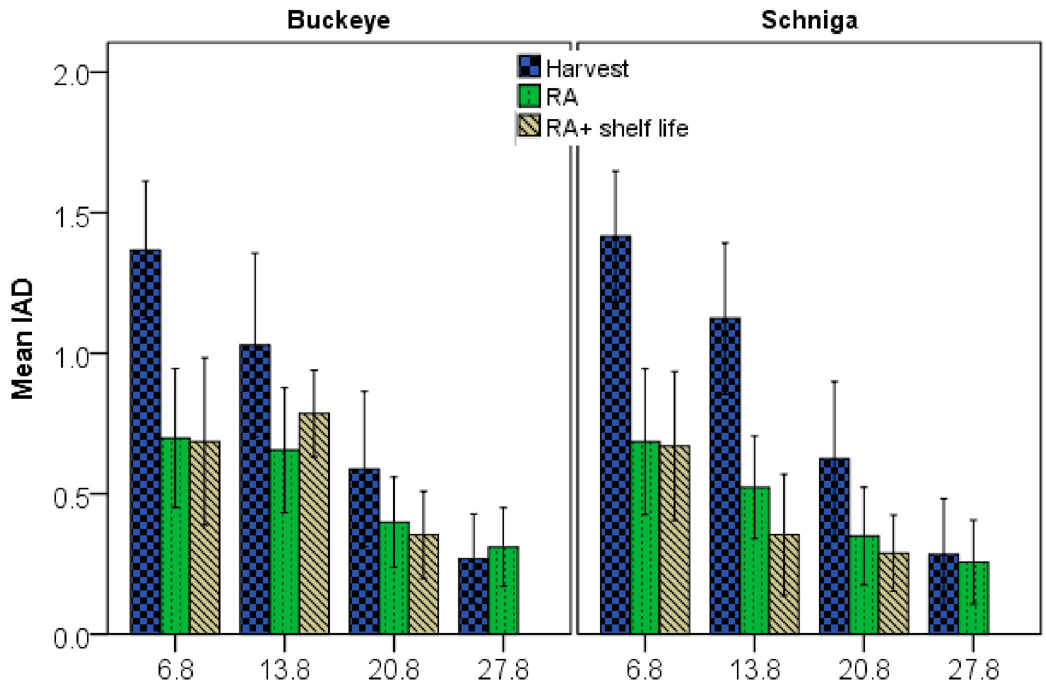
Figure 4. Sampling distribution during maturation (31 July to 4 September) of (**A**) IAD on the shaded fruit side and (**B**) starch pattern index (SPI 5), (**C**) fruit flesh firmness (FFF), of cv. 'Gala Buckeye' apples in 2017.

The fruit side did not show a significant ($p > 0.05$) effect on the detected IAD values within one sample (Tables A1 and A2). Similarly, no significant ($p > 0.05$) differences were observed in any of the monitored parameters between inner and outer canopy fruit (Tables A1 and A2).

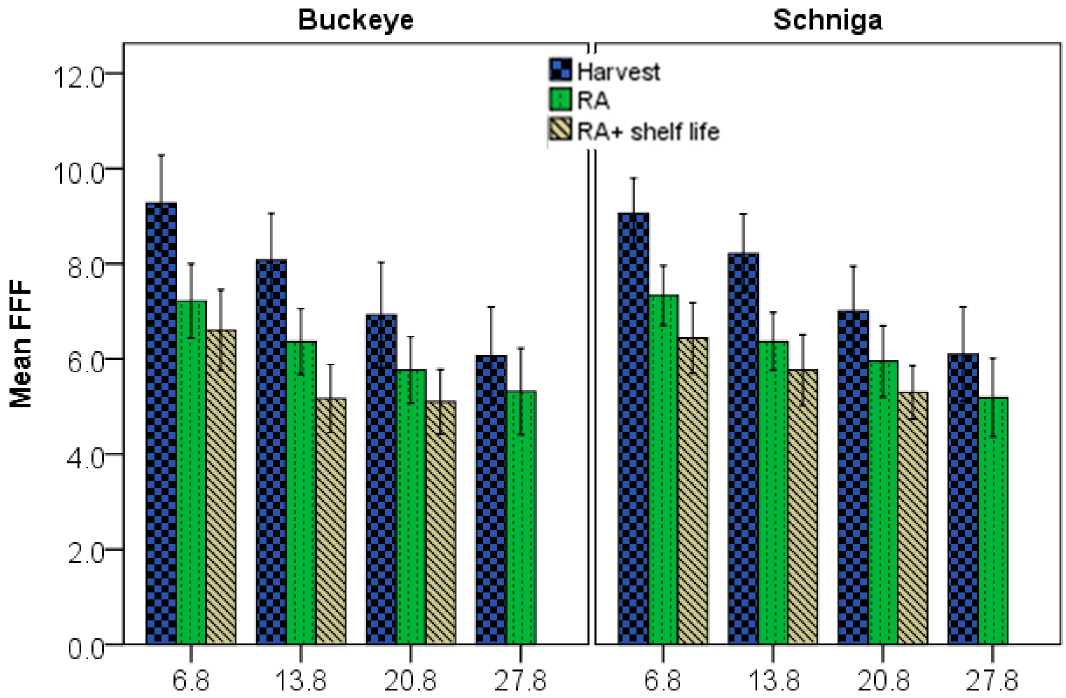
3.2. Kinetics of IAD in Relation to Starch Pattern Index and Fruit Flesh Firmness during Cold Storage

After 2.5 months of RA storage, only performed in 2015, the detected IAD values were significantly ($p < 0.005$) lower when compared to the ones that were observed at harvests for all but fruit picked on the 27 August, regardless of the canopy position or clone. Depending on the clone, while considering all four harvests, IAD changed in the range from 36 to 44% in the outer-canopy fruit and from 42 to 51% in the inner-canopy fruit, with the lower values being detected in the clone Buckeye (Figure 5A, Tables A3 and A4). During shelf life, no further significant decrease of IAD was observed (Figure 5A, Tables A3 and A4), contrary to the expectations, as studies have shown an acceleration of the chlorophyll degradation [38] and IAD [35] with increasing temperature in apple fruit.

Like IAD, FFF decreased during storage, but at a different rate (Figure 5B): A significant softening ($p < 0.05$) in the range of ~20% was observed, regardless of the clone or the canopy position (Tables A3 and A4). The trend of fruit softening that was observed during storage in the present study is in agreement with previous studies done on cv. 'Gala' [32]. In all cases, the softening of stored fruit exceeded the human differential perception limit [39]. The subsequent softening during shelf life was not relevant in terms of human perception [39].



(**A**)



(**B**)

Figure 5. Changes (mean and standard deviation) during 2.5 months of regular atmosphere (RA) storage and subsequent seven days of shelf life for the 4 harvests, with the optimal harvest date at 13 Augus 2015. The dynamics is shown for inner and outer canopy fruit of cv. 'Gala Buckeye' in 2015 for (**A**) IAD and (**B**) fruit flesh firmness in kgF (FFF).

3.3. IAD as a Tool for Assessing the Optimal Harvest Window

In both seasons, mean IAD in the fruit picked at optimal maturity ranged between 0.8–1.2, depending on the position of the fruit within the canopy and the fruit side (Tables A1 and A2). These values are higher than the recommendations that are based on relating IAD with SPI reported in the literature, where an IAD value of 0.4 was reported at optimal harvest for cv. 'Gala' apples grown in Canada [34] and in the Valtellina area in Italy [29], or values of 0.6–0.9 for cv. 'Gala' grown in Piedmont in Italy [29]. The notably differing climatic conditions between the growing regions, which have been shown to affect the accumulation and degradation of chlorophyll in the apple peel, offer a plausible explanation for the apparent discrepancies in the recommended IAD ranges. This explanation is also supported by the orchard- and/or altitude-specific range of IAD at optimal harvest in cv. 'Braeburn' and 'Nicoter' [40].

Significantly ($p > 0.005$) strong correlations in the magnitude of $r > |0.8|$ were observed between IAD, FFF, and SPI during fruit maturation. These results are in line with preceding studies on apple fruit, where relevant correlations were revealed between IAD and SPI [25,28–30], as well as IAD and FFF in some [20,28,30,32] but not all [20,29,32] apple cultivars.

Nevertheless, the observed strong ($r > |0.8|$) correlations that were observed between IAD, FFF, and SPI at harvest do not necessarily translate into IAD being able to predict either SPI or FFF, as exemplified by the results that were obtained by linear regression, identified as the best fit for the data according to the 'Curve Fit' procedure in SPSS (Tables A5–A8). When considering both clones, seasons, canopy positions, and fruit sides, IAD could significantly ($p < 0.01$) predict SPI, with the predictive power (r^2) between 0.7 to 0.9, and the standard error of the estimate in the range of 0.46 to 0.59 (Tables A5 and A7). This means that the maximal prediction error would translate into approximately 1 fifth of the SPI scale, in this case leading to an inaccurate determination of optimal maturity.

IAD could also significantly ($p < 0.01$) predict FFF, with the percentage of explained variability of FFF ranging between approximately 60 to 70% and a standard error of the estimate in the range of 0.8 kgF and 1.05 kgF. The potential maximal error is about 3 to 3.5 times higher than the human perception threshold for firmness [39].

In a commercial setting, for practical reasons of time and resource limitations, it is very likely that means of batches, rather than individual values, would be used for making predictions. By simulating such a system, on average, the predictive power of IAD for estimating SPI and FFF amounted to 0.9 ($p < 0.01$), with a standard error of the estimate in the range of around 0.15 units and 0.2 kgF for SPI and FFF. The absolute differences of FFF and SPI values among individual fruit cannot be estimated by simulating commercial production. Nevertheless, such strong predictions might already be usable under practical conditions, provided that a large number of IAD measurements is performed. Given the non-destructive nature, the operating speed, and ease-of-use of the DA meter, measuring large samples should be feasible, even in a commercial environment.

3.4. IAD as a Tool for Assessing Post-Storage Firmness

Mean IAD values that were obtained per batch at harvest were correlated to mean values of FFF measured after 2.5 months of RA and subsequent seven days shelf life to evaluate the potential of IAD for not only assessing the onset of the optimal harvest window, but also for estimating post-storage quality evolution (Table 1). Analyzing the data in terms of average was chosen in order to simulate real-life conditions, where tracking parameters back to individual fruit might not be feasible. Significant ($p < 0.005$) and strong ($r^2 > 0.8$) correlations were observed between mean IAD measured at different harvests and post-storage firmness readings (Table 1).

Table 1. Correlation (Pearson r) between mean IAD or starch pattern index (SPI) assessed at harvest and mean firmness (FFF) after storage (0 and 7 d shelf life) of two clones of cv. 'Gala'. Correlations are significant at $p < 0.005$ level (Tukey HSD, $p < 0.005$).

Clone	IAD vs. FFF after Storage	IAD vs. FFF after Shelf Life	SPI vs. FFF after Storage	SPI vs. FFF after Shelf Life
Buckeye	0.96	0.93	−0.94	−0.90
Schniga	0.94	0.95	−0.92	−0.96

These results are in agreement with the study of Nyasordzi et al. [30], who reported high correlations ($r^2 > 0.7$) between IAD readings at harvest and FFF after storage on different apple cultivars. Interestingly, the strength of correlations that were obtained between IAD at harvest and FFF after storage only slightly differed from the correlations obtained between SPI and post-storage FFF (Table 1). Thus, IAD and SPI were both able to predict post-harvest FFF with a similar power of prediction. In fact, IAD was considered to be a more stable indicator of storage potential over seasons when compared to SPI [41].

3.5. Potential of IAD for Practical Applications: Results of the Study Conducted in a Commercial Storage

As already observed earlier, the cv. Gala apples examined in a commercial storage revealed a very high variability in both IAD and FFF. IAD revealed a mean of 0.36 ± 0.26 on the sun-exposed and a mean of 0.52 ± 0.26 on the shaded fruit side. FFF showed very little differences with regards to fruit exposition, with both sides having mean values of 6.3 kgF ± 0.9. A contrasting situation, with higher IAD values being detected on the sun-exposed fruit side of cv. 'Gala' apples was detected in the study that was carried out at our research facilities, and also reported by other researchers [29]. One explanation for the lower values that were observed on the sun-exposed fruit side might be due to excessive-irradiation related chlorophyll degradation [29]. However, the lower IAD recorded on sun-exposed sides of the fruit might simply be explained as a measuring error since the sun-exposed and shaded fruit sides could not always be reliably distinguished in the already harvested fruit.

Contrary to the results of the experiments that were conducted under controlled conditions at our Research Centre, the data measured on fruit from the commercial storage did not yield strong correlations (Table 2), neither when individual values and nor when the means of both sides were correlated.

Table 2. Correlation (Pearson r) between IAD assessed at harvest and firmness (FFF) measured after storage of different cv. 'Gala' clones from different orchards. Correlations are significant at $p < 0.005$ level (Tukey HSD, $p < 0.005$).

Measuring Position	FFF Blush	FFF Shade	Mean of FFF Blush + Shade Per Apple
IAD blush	0.30	0.40	0.39
IAD shade	0.30	0.31	0.34
Mean of IAD blush+shade	0.33	0.38	0.39

In the commercial setting, the IAD failed to predict post-harvest FFF in any meaningful way. The fact that different batches of apples originated from different orchards and thus different environmental conditions offers one explanation as to why the correlations between IAD and FFF were so low. In addition, the apples were delivered by many different growers, likely using different agrotechnical measures, which might also influence the relationship between fruit skin chlorophyll and firmness. Whatever the reason, the fact remains that IAD cannot be used indiscriminately for assessing post-storage fruit firmness. Thus, its use remains limited to estimating fruit physiological stages under controlled conditions, at least for the moment.

4. Conclusions and Outlook

In all cases investigated, the broad range of maturity stages that were included in the study allowed for recognizing a sigmoidal time-course with a pronounced linear phase for IAD, SPI, and FFF, followed by an asymptotic trend only for IAD and SPI. These observations suggest that the changes in IAD were synchronized with SPI, but not with FFF.

By knowing IAD measured at harvest, significantly powerful estimations on individual fruits of the optimal harvest maturity, expressed as SPI ($r^2 > 0.7$, $p < 0.005$), and of the FFF not only at harvest but also after RA storage ($r^2 > 0.6$, $p < 0.005$) could be made. However, with the standard error of prediction ranging between 0.46 to 0.59 units for SPI and 0.82 to 1.1 kgF FFF, depending on the observed cases, the predictions were not accurate enough for practical applications.

The presence of a very high variability, which is common for fruits, was the denominator that all of the parameters had in common. As the presence of high biological variation can completely mask the underlaying dynamics, it is important that a large number of apples is analyzed [34], utilizing mean values per orchard for a practicable estimation.

In one orchard and over two seasons, mean IAD showed relative stability with values at optimal maturity ranging from 0.8–1.2 over clones, canopy position, and fruit sides. However, the mean values were characterized by high variability. This range overlaps with the mean IAD range of 1–1.2, recorded at harvest for fruit with the best storability and overall quality after 2.5 months in RA storage.

The investigation that was done under real-life conditions in a commercial fruit storage facility, with 69 batches typical for the region, showed that IAD measured at harvest did not indiscriminately correlate with post-storage FFF for cv. 'Gala' originating from different orchards. This proves, as reported elsewhere [28,29,32], that no absolute, universally valid IAD values characterizing SPI or FFF over growing regions, seasons, or productive practices exist.

Thus, IAD seems not in a position of replacing standard maturity parameters, but can serve as a supplementary index, providing additional information regarding fruit physiological status. However, mean IAD values that were obtained at optimal maturity from the same orchards remained stable over seasons, which strongly suggests that, provided the calibrations and validations are not only cultivars, but also orchard-specific, IAD has the potential for estimating maturity and storability of apple fruit. To test this potential, multiannual studies need to be done per cultivar, location and production practices. Combining IAD and conventional quality parameters also could be promising. Furthermore, a study relating IAD values with consumer acceptance and quality perception of cv. 'Gala' on the market, relevant to the producers in the studied region, could also be of interest, as consumers significantly differ from country to country.

Author Contributions: Both authors contributed to the manuscript and have approved the final manuscript.

Funding: The presented work was carried out in the framework of the project 'Monitoring key environmental parameters in the alpine environment involving science, technology and application' (MONALISA), funded by the Autonomous Province of Bolzano (Italy).

Acknowledgments: We wish to thank members of the Postharvest Department at Laimburg for their skillful collaboration and technical assistance and Martin Thalheimer for proofreading.

Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

Table A1. Changes of IAD and standard maturity parameters in cv. 'Gala' apples during ripening in season 2015. The measurements (mean and standard deviation) of IAD were carried out on sun-exposed fruit side, with all other measurements carried out on the transition between the sun-exposed and shaded fruit side.

Clone	Assessment	Position	IAD	SPI 5	FFF (kgF)	SSC (Brix)	TA(g/L)
Schniga	6 August	inner	1.5 ± 0.1	1.7 ± 0.2	9.3 ± 0.6	8.4 ± 0.4	3.8 ± 0.0
		outer	1.3 ± 0.3	2.2 ± 0.5	8.8 ± 0.8	9.7 ± 0.7	3.4 ± 0.1
	10 August	inner	1.3 ± 0.2	1.8 ± 0.4	8.9 ± 0.6	9.3 ± 7.0	3.6 ± 0.1
		outer	1.2 ± 0.2	2.0 ± 0.5	8.7 ± 1.0	10.1 ± 0.6	3.5 ± 0.1
	13 August	inner	1.2 ± 0.2	2.4 ± 0.5	8.3 ± 0.9	9.9 ± 0.6	3.5 ± 0.1
		outer	1.0 ± 0.3	2.8 ± 0.5	8.2 ± 0.8	10.8 ± 0.6	3.2 ± 0.1
	17 August	inner	0.8 ± 0.3	3.2 ± 0.6	7.4 ± 0.7	10.2 ± 0.6	3.1 ± 0.0
		outer	0.7 ± 0.2	3.3 ± 0.7	7.2 ± 1.0	11.0 ± 0.6	3.0 ± 0.1
	20 August	inner	0.6 ± 0.3	4.0 ± 0.7	7 ± 1.0	10.7 ± 0.4	3.1 ± 0.1
		outer	0.7 ± 0.3	3.5 ± 0.8	7 ± 0.9	11.2 ± 0.6	3.3 ± 0.1
	24 August	inner	0.3 ± 0.2	4.4 ± 0.6	6.3 ± 0.8	10.9 ± 0.5	2.9 ± 0.1
		outer	0.3 ± 0.2	4.2 ± 0.6	6.4 ± 0.9	11.8 ± 0.5	3.0 ± 0.1
	27 August	inner	0.3 ± 0.2	4.6 ± 0.7	6.1 ± 1.0	11.1 ± 0.5	3.1 ± 0.1
		outer	0.3 ± 0.2	4.5 ± 0.5	6.1 ± 1.0	11.6 ± 0.4	3.2 ± 0.2
	31 August	inner	0.2 ± 0.2	4.8 ± 0.2	5.4 ± 0.8	11.5 ± 0.5	2.9 ± 0.2
		outer	0.2 ± 0.1	4.7 ± 0.4	5.6 ± 0.9	11.8 ± 0.4	2.8 ± 0.1
Buckeye	6 August	inner	1.5 ± 0.1	1.6 ± 0.2	9.7 ± 0.7	8.6 ± 0.6	4.0 ± 0.1
		outer	1.3 ± 0.3	2.9 ± 0.7	8.8 ± 1.1	9.8 ± 0.7	3.6 ± 0.2
	10 August	inner	1.4 ± 0.1	1.7 ± 0.3	9.4 ± 0.8	9.1 ± 0.5	4.0 ± 0.3
		outer	1.2 ± 0.3	2.5 ± 0.7	8.6 ± 1.2	10.5 ± 0.6	3.5 ± 0.2
	13 August	inner	1.1 ± 0.3	2.4 ± 0.3	8.2 ± 0.8	9.9 ± 0.5	3.6 ± 0.1
		outer	1.0 ± 0.4	2.8 ± 0.5	7.9 ± 1.1	10.8 ± 0.9	3.4 ± 0.0
	17 August	inner	0.8 ± 0.3	3.1 ± 0.8	7.5 ± 1.0	10.3 ± 0.5	3.5 ± 0.1
		outer	0.7 ± 0.3	3.2 ± 0.6	7.4 ± 0.9	11.3 ± 0.8	3.1 ± 0.2
	20 August	inner	0.6 ± 0.3	3.7 ± 0.6	7.3 ± 1.3	10.8 ± 0.6	3.2 ± 0.1
		outer	0.6 ± 0.2	3.9 ± 0.7	6.6 ± 0.8	11.7 ± 0.6	3.0 ± 0.1
	24 August	inner	0.4 ± 0.2	4.3 ± 0.6	6.3 ± 0.8	11.0 ± 0.5	3.0 ± 0.0
		outer	0.4 ± 0.2	4.2 ± 0.6	6.5 ± 1.0	11.8 ± 0.6	3.2 ± 0.0
	27 August	inner	0.3 ± 0.2	4.4 ± 0.5	6.3 ± 1.0	11.5 ± 0.4	3.1 ± 0.1
		outer	0.3 ± 0.1	4.6 ± 0.5	5.9 ± 1.0	11.7 ± 0.5	3.0 ± 0.1
	31 August	inner	0.2 ± 0.1	4.6 ± 0.4	5.9 ± 0.9	11.3 ± 0.7	2.9 ± 0.3
		outer	0.2 ± 0.1	4.5 ± 0.4	6.2 ± 0.9	12.1 ± 0.6	3.0 ± 0.2

Table A2. Changes of IAD on blush and shade side of each fruit and standard maturity parameters (mean and standard deviation) in cv. 'Gala' apples during ripening in season 2017.

Clone	Assessment	IAD Blush	IAD Shade	SPI 5	FFF (kgF)	SSC (Brix)	TA(g/L)
Schniga	31 July	1.5 ± 0.2	1.3 ± 0.1	1.4 ± 0.3	9.6 ± 0.7	8.9 ± 0.6	3.5 ± 0.3
	3 August	1.3 ± 0.3	1.1 ± 0.2	1.9 ± 0.6	8.7 ± 0.9	9.4 ± 0.6	3.2 ± 0.1
	7 August	1.0 ± 0.4	0.8 ± 0.2	2.4 ± 1.0	8.3 ± 1.0	9.9 ± 0.8	3.2 ± 0.1
	10 August	0.9 ± 0.3	0.8 ± 0.2	3.2 ± 0.8	7.8 ± 0.9	10.6 ± 0.8	3.1 ± 0.1
	17 August	0.7 ± 0.3	0.5 ± 0.3	3.6 ± 0.8	7.6 ± 0.9	11.5 ± 0.7	2.6 ± 0.4
	21 August	0.4 ± 0.2	0.3 ± 0.2	4.4 ± 0.6	7.3 ± 0.9	11.9 ± 0.6	2.5 ± 0.1
	25 August	0.4 ± 0.2	0.2 ± 0.2	4.7 ± 0.4	6.7 ± 1.0	11.8 ± 0.6	2.9 ± 0.2
	28 August	0.3 ± 0.1	0.1 ± 0.1	4.8 ± 0.3	6.6 ± 1.0	11.7 ± 0.5	2.6 ± 0.1
	31 August	0.3 ± 0.1	0.1 ± 0.1	4.9 ± 0.1	6.7 ± 1.1	11.8 ± 0.6	2.1 ± 0.4
	4 September	0.2 ± 0.1	0.0 ± 0.0	5 ± 0.0	5.8 ± 0.8	11.5 ± 0.7	1.9 ± 0.1
Buckeye	31 July	1.4 ± 0.1	1.2 ± 0.1	1.4 ± 0.2	9.4 ± 0.8	8.3 ± 0.4	3.9 ± 0.1
	3 August	1.2 ± 0.3	1.1 ± 0.2	1.9 ± 0.6	8.9 ± 0.8	9.1 ± 0.7	3.7 ± 0.1
	7 August	1.0 ± 0.3	1.0 ± 0.2	2.0 ± 0.3	8.9 ± 0.7	9.6 ± 0.8	3.5 ± 0.1
	10 August	0.8 ± 0.3	0.8 ± 0.3	2.8 ± 0.8	8.1 ± 1.0	10.0 ± 0.8	2.9 ± 0.3
	17 August	0.6 ± 0.2	0.4 ± 0.2	3.6 ± 0.5	7.6 ± 1.0	11.0 ± 0.5	2.7 ± 0.2
	21 August	0.4 ± 0.1	0.3 ± 0.2	4.0 ± 0.6	7.3 ± 0.7	10.9 ± 0.6	2.8 ± 0.4
	25 August	0.3 ± 0.1	0.2 ± 0.1	4.6 ± 0.4	7.1 ± 0.9	11.8 ± 0.7	3.2 ± 0.1
	28 August	0.3 ± 0.1	0.1 ± 0.1	4.7 ± 0.4	7.0 ± 1.1	11.5 ± 0.6	3.1 ± 0.0
	31.August	0.2 ± 0.1	0.1 ± 0.1	4.8 ± 0.5	6.8 ± 1.0	11.9 ± 0.6	2.5 ± 0.3
	4 September	0.2 ± 0.1	0.1 ± 0.2	4.8 ± 0.4	6.8 ± 1.0	11.7 ± 0.8	2.5 ± 0.4

Table A3. Post-storage values of IAD and fruit flesh firmness-FFF (mean and standard deviation) of cv. 'Gala Schniga' apples harvested at different maturity stages.

Assessment	Position	Storage in Months	IAD	FFF (kgF)
6 August	inner	2.5	0.7 ± 0.3	7.3 ± 0.5
		2.5 + 7d shelf life	0.7 ± 0.3	6.5 ± 0.6
	outer	2.5	0.6 ± 0.2	7.4 ± 0.7
		2.5 + 7d shelf life	0.6 ± 0.2	6.3 ± 0.8
13 August	inner	2.5	0.5 ± 0.1	6.4 ± 0.6
		2.5 + 7d shelf life	0.4 ± 0.2	5.8 ± 0.8
	outer	2.5	0.5 ± 0.2	6.3 ± 0.6
		2.5 + 7d shelf life	0.4 ± 0.2	5.7 ± 0.8
20 August	inner	2.5	0.4 ± 0.2	6.1 ± 0.8
		2.5 + 7d shelf life	0.3 ± 0.1	5.1 ± 0.6
	outer	2.5	0.4 ± 0.2	5.8 ± 0.7
		2.5 + 7d shelf life	0.3 ± 0.2	5.5 ± 0.5
27 August	inner	2.5	0.2 ± 0.1	5 ± 0.8
		2.5 + 7d shelf life	NA ± NA	NA ± NA
	outer	2.5	0.3 ± 0.1	5.3 ± 0.8
		2.5 + 7d shelf life	NA ± NA	NA ± NA

Table A4. Post-storage values of IAD and fruit flesh firmness-FFF (mean and standard deviation) of cv. 'Gala Buckeye' apples harvested at different maturity stages.

Assessment	Position	Storage in Months	IAD			FFF (kgF)		
6 August	inner	2.5	0.8	±	0.2	7.3	±	0.6
		2.5 + 7d shelf life	0.8	±	0.3	6.8	±	0.5
	outer	2.5	0.6	±	0.2	7.1	±	0.9
		2.5 + 7d shelf life	0.6	±	0.2	6.4	±	1.0
13 August	inner	2.5	0.7	±	0.2	6.3	±	0.7
		2.5 + 7d shelf life	0.5	±	0.2	5.8	±	0.7
	outer	2.5	0.6	±	0.2	6.5	±	0.7
		2.5 + 7d shelf life	0.5	±	0.3	5.2	±	0.6
20 August	inner	2.5	0.4	±	0.2	5.7	±	0.7
		2.5 + 7d shelf life	0.4	±	0.2	5.1	±	0.7
	outer	2.5	0.4	±	0.1	5.9	±	0.7
		2.5 + 7d shelf life	0.4	±	0.2	5.1	±	0.6
27 August	inner	2.5	0.3	±	0.2	5.1	±	0.9
		2.5 + 7d shelf life	NA	±	NA	NA	±	NA
	outer	2.5	0.3	±	0.1	5.5	±	0.9
		2.5 + 7d shelf life	NA	±	NA	NA	±	NA

Table A5. Summary of linear regression for predicting SPI based on IAD measurements for season 2015. The regression was significant at $p > 0.005$.

Clone	Canopy	r^2	SEE
Buckeye	inner	0.8	0.54
	outer	0.7	0.59
Schniga	inner	0.8	0.54
	outer	0.8	0.52

Table A6. Summary of linear regression for predicting SPI based on IAD measurements for season 2017. The regression was significant at $p > 0.005$.

Clone	IAD	r^2	SEE
Buckeye	blush	0.8	0.57
	shaded	0.9	0.46
Schniga	blush	0.8	0.58
	shaded	0.9	0.48

Table A7. Summary of linear regression for predicting FFF based on IAD measurements for season 2015. The regression was significant at $p > 0.005$.

Clone	Canopy	r^2	SEE
Buckeye	inner	0.9	0.86
	outer	0.7	1.06
Schniga	inner	0.9	0.82
	outer	0.8	0.88

Table A8. Summary of linear regression for predicting FFF based on IAD measurements for season 2017. The regression was significant at $p > 0.005$.

Clone	IAD	r^2	SEE
Buckeye	blush	0.6	0.89
	shaded	0.6	0.85
Schniga	blush	0.6	0.91
	shaded	0.6	0.89

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